POST-HARVEST TECHNOLOGY, PRESERVATION AND QUALITY OF FISH IN SOUTHEAST ASIA

NOVEMBER 13-17, 1989

Bangkok, Thailand.

Edited by:

P. J. A. REILLY

R. W. H. PARRY

and

L. E. BARILE

International Foundation For Science.

POST-HARVEST TECHNOLOGY, PRESERVATION AND QUALITY OF FISH IN SOUTHEAST ASIA

November 13-17, 1989
Bangkok, Thailand

Edited by:

P.J.A. REILLY
R.W.H. PARRY
and
L.E. BARILE

INTERNATIONAL FOUNDATION FOR SCIENCE

POST-HARVEST TECHNOLOGY, PRESERVATION AND QUALITY OF FISH IN SOUTHEAST ASIA

November 13-17, 1989
Bangkok, Thailand

ORGANISED BY: *International Foundation for Science*
National Research Council of Thailand
Mahidol University

Proceedings of the Workshop on Post-harvest Technology, Preservation and Quality of Fish in Southeast Asia

Published by:
International Foundation for Science
Grev Turegatan 19
S-114 38 Stockholm, Sweden

ISBN 91-85798-26-6

Printed by Echanis Press, Manila, Philippines

Table of Contents

of fish
work o
Scienc
present
contrib

cured f
The wo
for futu

the Nat

FISH FERMENTATION TECHNOLOGY - A REVIEW

Cheri-Ho Lee
Department of Food Technology, Korea University, Seoul, Korea

Summary

The historical background of fish fermentation in Asia and other regions of the world is reviewed. The classification of fermented fish products in different regions is attempted with respect to the technology involved. The fermented fish products are largely divided into three groups: (1) high-salt, (2) low- salt, and (3) non-salt fermented. High-salt fermented products contain over 20% of salt and are represented by fish sauce, cured fish and fish paste. Low-salt fermented products contain 6-18% salt and are subdivided into lactic fermented products with added carbohydrate and acid pickling associated with low temperature. Non-salt fermented products are represented by the solid state bonito fermentation and some alkaline fermentation of flat fishes. The local names of the products in different regions are compared and classified accordingly. The microbial and biochemical changes during fish fermentation are considered in relation to the quality of the products, and their wholesomeness is reviewed.

Introduction

Fish fermentation is an old technology used for the preservation of freshwater and marine animals, which are highly perishable and localised in production and seasonally fluctuating in catching (Ruddle, 1989). The technology appears to have evolved with the availability of salt and non-pastoral way of life. There is a strong correlation throughout the world between the use of fermented fish products and the use of cereals, especially rice, and vegetables (Ishige, 1989). Although the use of fermented fish product is nowadays mainly confined to East and Southeast Asia, traces of this technology can be found throughout old human civilisations.

Liquamen and garum was an important condiment in the cuisine of the Romans who had adopted it from the Greeks. It was made on a commercial scale at several sites around the Mediterranean from a variety of small fish including red mullet, sprats, anchovies and mackerel (Wilson, 1976; Adams, 1986). The Italian anchovy paste is a vestige of this old technology. In Northern Europe, salted fish was an important commodity, and around the end of the 12th century barrel-salted herrings became an important export item of many towns near the Baltic sea. The salted herrings were part of the daily menu in many places in Scandinavia. This has recently changed drastically, and the traditional wet-salted herrings are now mostly being used as raw materials for less salty, semi-preserved herring products which are sold as expensive delicacies (Knochel, 1989).

On the other hand, the East and Southeast Asian region preserves this traditional technology and benefits the people with the simple and materials-saving method of food supply. Ishige (1989) explains this as resulting from the dietary pattern of the people in this region. The main area for the consumption of fermented products coincides with the principal region of irrigated rice cultivation. Those parts of Asia where rice is the staple food are characterised by a low consumption of meat and dairy products but a high consumption of fish and fish products. The consumption of large quantities of rice, which is bland in taste, necessitates the use of side dishes which are small in quantity and highly salty. Fermented fish products and soybean products are the most suitable items for this purpose. They have the advantage of being simple to preserve and have a long shelf-life. They do not require elaborate cooking for each meal, and provide meaty flavour, amino acids, vitamins, and other minor nutrients needed in the diet.

Although it is difficult to preclude that fish fermentation technology has been developed independently in different regions of the globe, the Mekong Basin is most probably the place of origin of fermented fish products (Ishige, 1989). In the original form fish was probably salted for preservation, and the exuded liquid drained off and used as the condiment for rice meals, same as the ancient type of soysauce and soybean paste production. Later, soybean fermentation technology was developed in the Northeastern Asia, where soybean cultivation was possible, and fermented soybean products became the dominant condiment in this region. Figure 1 divides the two cultural regions of soysauce and fish sauce in Asia (Ishige, 1989; Lee, 1989).

Figure 1. Cultural area of fermented condiments in East Asia (Ishige, 1989; Lee, 1989)

Classification of fermented fish products

Since the most original form of fermented fish products was probably salted fish and the exuded liquid was used as fish sauce, fermented fish products are basically salt-fermented products. Depending on the amount of salt added, the products are classified into high-salt (> 20% salt of total weight), low- salt (6-18% salt) and no-salt products, as shown in Figure 2.

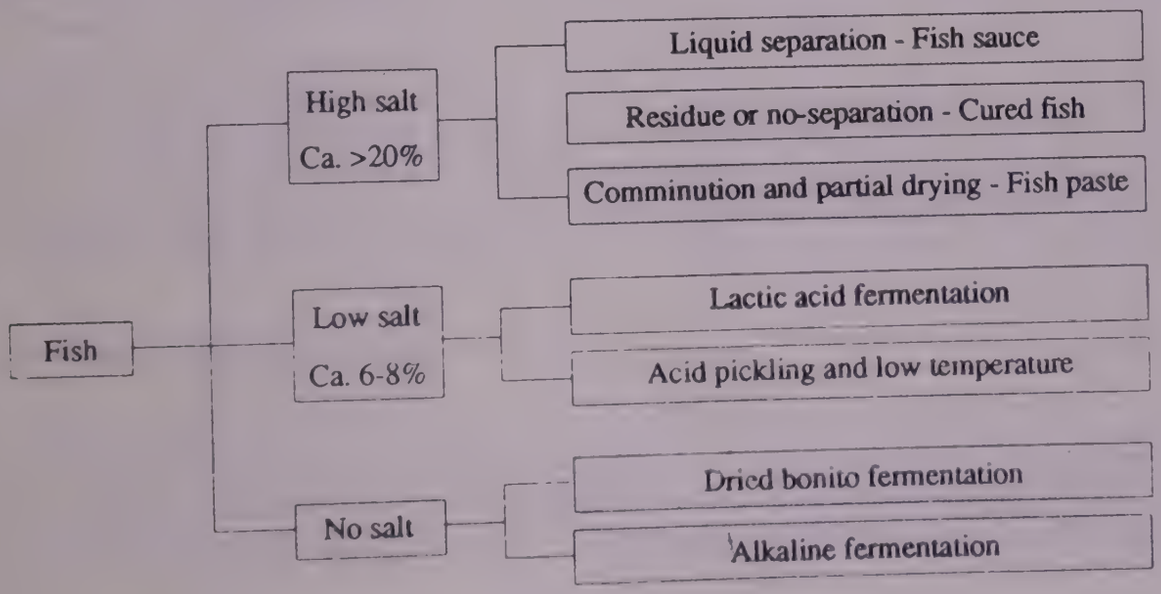

Figure 2. Classification of fermented fish products

When the salt concentration is higher than 20% of total weight, pathogenic and putrefactive microorganisms cannot grow and the product does not need other preservative means. The first criterion for the subdivision of this group is the degree of hydrolysis, which is influenced by fermentation time and temperature, added enzyme sources and the water content. The fully hydrolysed liquid is fish sauce. The name cured fish is confined here to represent the partially hydrolysed fish products which retain the original shape of fish immersed in the exuded liquid, and this form as such is frequently used as side dish for rice meals (Lee et al., 1987; Boon-Long, 1987). Fish paste is characterised in that the salted fish is partially dried in order to restrict the degree of hydrolysis and comminuted to produce the homogeneous, solid condiment. Each class can be further subdivided by the kind of raw materials, such as fish species, portion of fish, etc, and thus numerous kinds of products can be named. In Korea, in the category of cured fish, more than 30 kinds of products are produced (Lee, 1989).

When the salt concentration is lower than 20%, the salted fish undergoes rapid spoilage, and other means of preservation is needed. Lactic fermentation with added carbohydrate is an old method of fish preservation in low-salt processes.

Rice, millet, flour, and even syrup or sugar are used as the carbohydrate source. The amount of added carbohydrate and salt concentration primarily control the extent of acid fermentation and the keeping quality. An alternative method is keeping the low-salt fermented fish with added vinegar in low temperature. This method is widely practised in the Scandinavian countries (Knochel, 1989). Many of Asian countries produce salt cured and dried fish products, for example, Plaken in Thailand (Boon-Long, 1987), Jambalroti in Indonesia (Putro, 1989), Maldive fish in Sri Lanka (Subasinghe, 1989) and Gulbi in Korea (Kin et al., 1989) but the role of fermentation in these products is not fully understood.

Fish fermentation without added salt is not a common practice. In some local specialities, half-spoiled fish or alkaline fermentation in leafy plant ash is used (Souane, 1987a). The propagation of mould on the dried bonito (katsuobushi) processing in Japan is another example of non-salt fish fermentation (Kanazawa, 1986).

Three kinds of Chinese ideographs indicating fermented fish products appear in an old Chinese literature "Churai" written in the 3rd century; i.e. Hae, Ja and Chi (Chang, 1976). Hae is a fermented meat or fish made by mixing and aging with salt, and occasionally with added wine, Koji and Nuruk, an alcohol fermentation starter. A Chinese dictionary "Solmun" describes Hae as meat or fish sauce, Ja as acidic fermented fish. Chi is a fish sauce, which is considered quite similar to Hae but the detailed characteristics are not known. Adams (1986) classified fermented fish products into two groups; i.e. fish/salt products and fish/salt/carbohydrate products. A similar classification was also made by Ishige (1989).

Owens and Mendoza (1985) tried to divide fermented fish products on the basis of enzyme hydrolysed versus microbial fermented. They subdivided the products primarily involving enzymic hydrolysis into four groups; (1) hydrolysis in >20% salt, (2) hydrolysis in salt+drying, (3) hydrolysis at low temperature and (4) hydrolysis at low pH values. The products preserved by microbial fermentation are subdivided into two groups; (1) fermented with added carbohydrate, and (2) fermented without added carbohydrate. However, a large controversy exists on the role of microorganisms in high-salt fish fermentation.

Microbial fermentation vs enzymic hydrolysis

Great controversy still continues as to the name of fermented fish when high salt concentration is used. Many microbiologists prefer to use the term enzymic hydrolysed, instead, or "cured fish" because no microorganisms can grow in such a highly osmolytic condition, created in high-salt fish fermentation (Owens and Mendoza, 1985; Reilly and Barile, 1986). However, some recent findings support the possible role of microbial degradation in the formation of fermented fish flavour in high-salt fermentations.

Ito et al. (1989a) found that mysis and shrimp had a factor which increases halophilic characteristics of some halophilic bacteria. The halophilic characteristics of bacteria increased when they were grown in a medium with added extracts from digested shrimp or salt fermented mysis. They explained that these halophilic bacteria kept the halophilic characteristics by getting energy from other substrate rather than oxygen respiration of salt. It was suggested that halophilic bacteria gain energy through the activity of lactate oxidase linked cytochrome b, because this oxidase has halophilic character. By the addition of shrimp or mysis extract, they could grow *Paracoccus halodenitrificans* under the higher concentration of salt than had previously been thought possible.

Knochel (1989) pointed out the importance of selecting suitable methodologies for the microbiological examination of gh-salted foodstuffs. The environment in high-salt fermented fish product is extreme, with over 20% salt in the aqueous phase. ten counts are obtained on media with low salt content reflecting neither the quantity nor the composition of the active croflora. The cell of *P. halodenitrificans* plasmolyses under 12% brine (Ito et al., 1989a).

Many of the investigations on the microflora in high-salt fermented fish products indicate the growth of halophilic bacteria ring fermentation (Mheen, 1989; Lee and Choe, 1984; Chung and Lee, 1976; Ito et al., 1989b; Karim, 1989; Phithakpol, 1989; einkraus, 1983). Mheen (1989) demonstrated the favourable effect of starter culture, *Bacillus* species, on the ripening and vour formation of high-salt fermentation of anchovy.

It is still unclear the share of importance between microbial degradation and enzymic hydrolysis in high-salt fish rmentation. Therefore, it is reasonable to use the term fermentation for this process, which allows for the transformation of ganic substances into simpler compounds by the action of enzymes and microorganisms (Mackie et al., 1971). Ishige (1989) ade the distinction between salting and fermentation. The objective in salting fish is to retain the shape of the fish and prevent ecay, and thereby to produce a preserved cooking ingredient akin to original raw material, whereas the intent of fermentation to create a material that does not exist in nature. In this context, it can also be added to the definition of fermentation that it can e a process by which inedible raw materials are made edible without cooking by the action of microorganisms.

ynonyms and variations

Comparing the products of different regions, identical but differently named products are frequently found, and this tuation creates confusion and difficulties in communication. Table 1 shows the synonyms found in high-salt fermented fish roducts. Most of the countries in the East and Southeast Asia have fish sauce, but it varies in the flavour, physical properties nd the raw materials used.

Table 1. Classification of high-salt fermented fish products in different countries

Country	Fish sauce	Cured fish	Fish paste
Burma	Ngan-pya-ye	-	Ngapi
Cambodia	Nuoc-mam	-	Prahoc
China	Yu-lu	-	-
Indonesia	Ketjap-ikan	Pedah	Trassi
Japan	Shotsuru	Shiokara	-
Korea	Jeot-Kuk	Jeotkal	-
Malaysia	Budu	-	Belacan
Philippines	Patis	Bagoong	Bagoong
Sri Lanka	Blood pickle	Jaadi	-
Thailand	Nampla	-	Kapi
Vietnam	Nuoc-mam	-	Mam-ca

Figure 3 shows the general processing procedure for fish sauce and cured fish. Depending on the degree of hydrolysis r fermentation time and the separation method, two types of sauce, namely, clear and turbid, are produced. Ngan-pya-ye, Nuoc-nam, Nampla, Shotsuru and Yu-lu are clear type fish sauces, while Budu, Patis, Ketjap-ikan and Jeot-kuk are turbid. Some of ne turbid sauces are obtained from the exuded liquid of cured fish, for example, Patis from Bagoong production in the Philippines nd Jeot-kuk from Jeot-kal production in Korea. In the Northeastern Asia cured fish products are more important than fish paste. ish paste, especially those made from shrimp and planktonic animals such as Seinsa Ngapy, Belacan, Trassi, Prahoc and Kapi, re important in Southeastern Asian diets. Figure 4 shows the general processing procedure of fish paste. For the more elaborated uality improvement, partial drying and packed fermentation can be repeated.

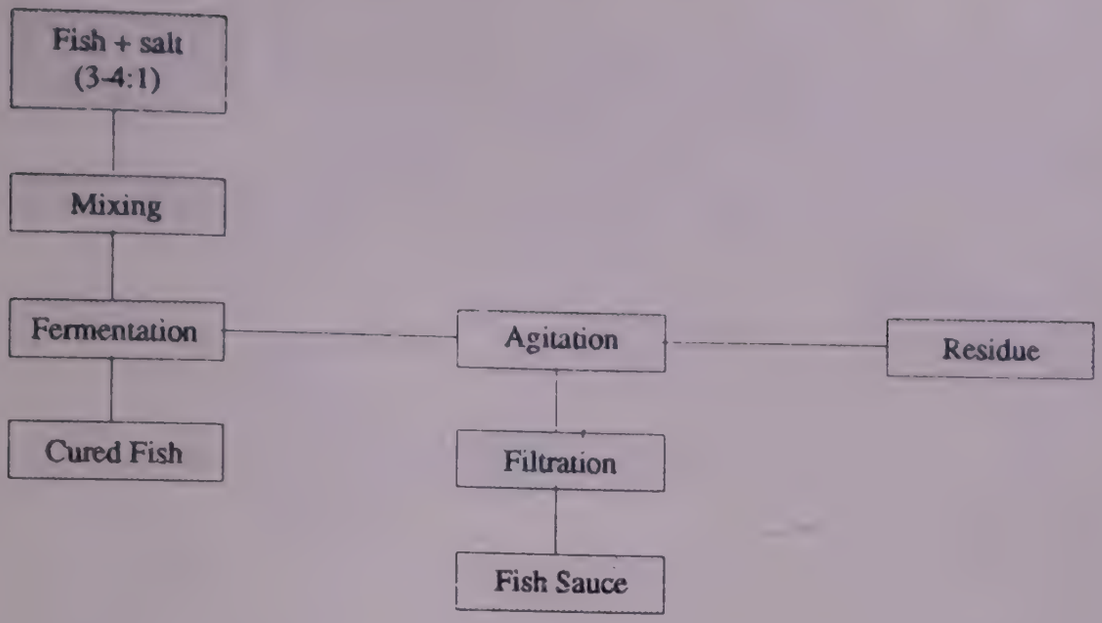

Figure 3. General processing flow of cured fish and fish sauce

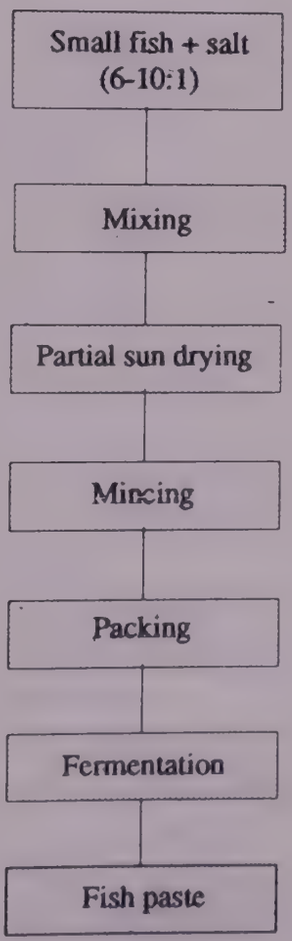

Figure 4. General processing flow of fish paste

Table 2 summarises the low-salt fermented fish products in different countries. In Scandinavia most of the traditional low-salt fermented fish products are transformed into pickled products in vinegar. And these products generally require low temperature storage.

Table 2. Classification of low-salt fermented fish products in different countries

Country	Lactic fermented	Acid pickling
Norway	Rakeorret	-
England	-	Tidbits
Germany	-	Schnell-maatjes
Japan	Narezushi	-
Korea	Sikhae	-
Malaysia	Bekasam	-
Philippines	Burong-isda, Balao-balao	-
Thailand	Pla-som, Pla-ra, Pla-chom	-
Vietnam	Mum-tom	-

On the other hand, most of Asian products are lactic fermented with added cereals, as shown in Figure 5. Rice, either cooked or roasted, is the most frequently used carbohydrate source, but other sources such as millet in Sikhae are also used (Lee et al., 1983). In some cases fruits and vegetables, for example tamarind in Bekasam for the reduction of pH, and garlic and pepper in Sikhae, are added. The antimicrobial effect of garlic to some putrefactive microorganisms such as *Bacillus* in lactic fermented fish products has been demonstrated (Souane et al,, 1987).

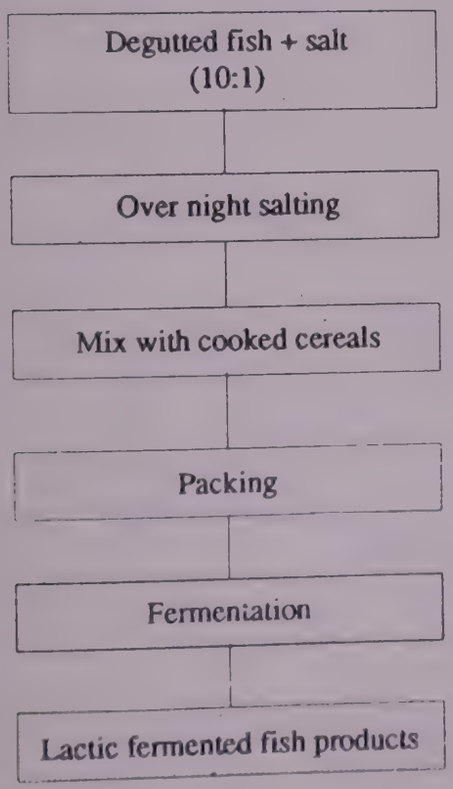

Figure 5. General processing flow diagram of lactic fermented fish products

Microbial and biochemical changes during fish fermentation

Figure 6 shows the microbial and biochemical changes of a typical high-salt cured fish made from anchovy (Lee et al., 1986). The salt content of the product was 20% (w/w) of total weight. The total number of viable cell increased during the first 40 days and then decreased. This was mainly attributed to the growth of *Pediococcus* and *Halobacterium*. The concentrations of soluble-N and animo-N increased steadily during the first 60 days and this coincided with the development of optimum taste. The volatile basic-N content increased in two steps and the second step coincided with the start of taste deterioration and this was also related to the maximum growth of yeast in the system.

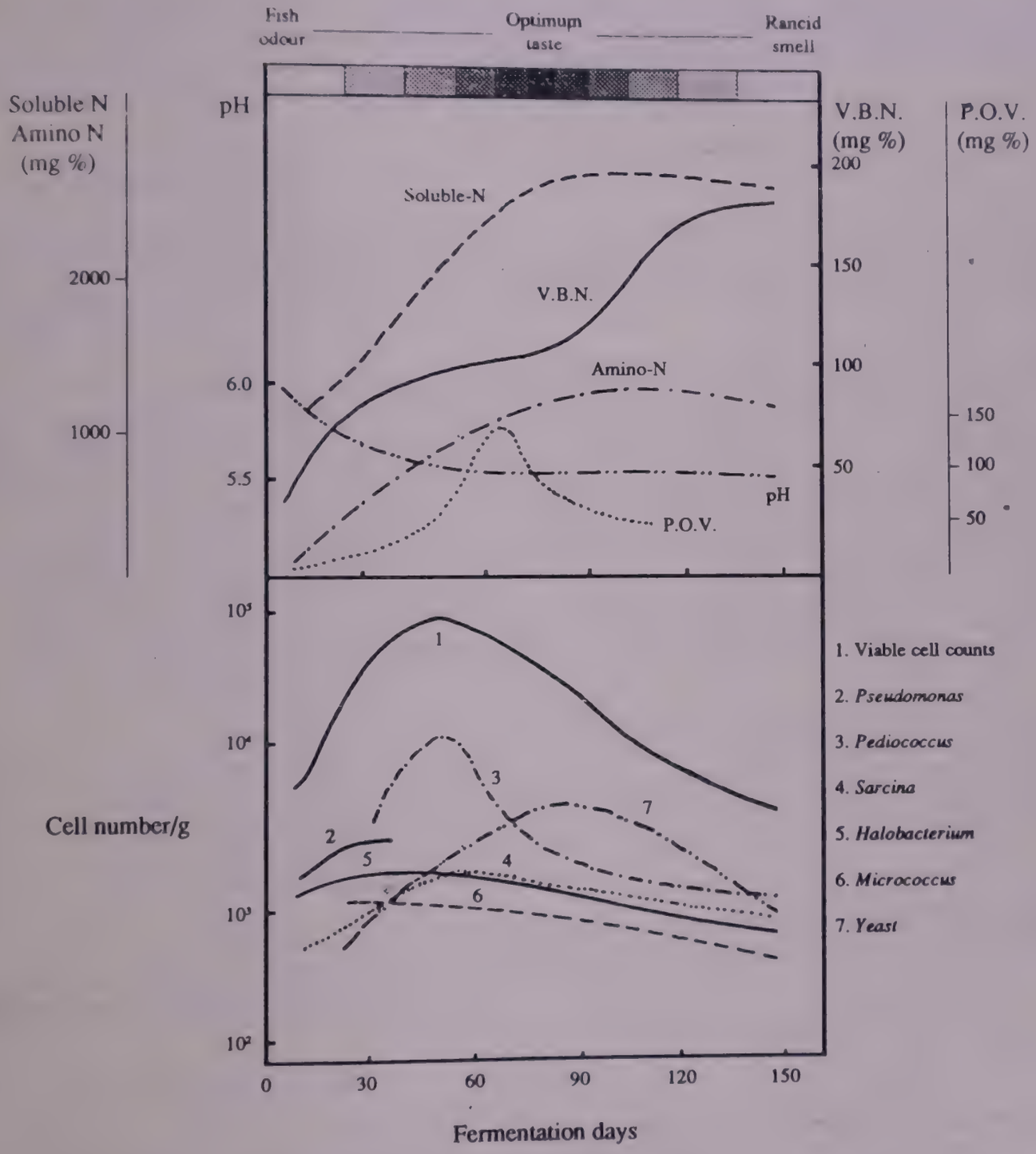

Figure 6. Microbial and biochemical changes during the fermentation of a cured fish, anchovy Joet-kal (Lee et al., 1986)

Figure 7 shows the microbial and biochemical changes of a typical lactic fermented fish product incubated at 20°C. The salt concentration of the product was 8% (w/w) of total weight and cooked millet was the carbohydrate source and garlic and red pepper powder were added (Lee, 1983; Lee et al., 1986). The pH decreased rapidly during the first 3-5 days from 6.5 to below 5.0 and texture softening took place from 3-4 days after fermentation. The amino-N concentration increased steadily up to 14 days and this coincided with the attainment of the optimum taste. The number of lypolytic bacteria decreased rapidly during the initial stage of fermentation and the proteolytic bacteria increased until 12 days of fermentation and thereafter decreased rapidly. The acid forming bacteria increased rapidly and became the dominating microorganisms in one week of the fermentation and reached the maximum at 16 days of fermentation. In this case, the taste deterioration was also associated with the maximum growth of yeast and acid-forming bacteria.

The important bacteria for the lactic fermentation were identified as *Leuconostoc mesenteroides* and *Lactobacillus plantarum* (Souane, 1987b). The role of these acid forming bacteria for the preservation of fish is apparent, but a more important aspect is their ability to produce acceptable flavour during the fermentation. The mechanism of the production of palatable flavour components by these acid forming bacteria in cereal substrate is presently under investigation.

Wholesomeness of fermented fish products

The major potential hazard associated with proteinaceous foods like fermented fish is from the growth of food poisoning bacteria, presence of parasitic worms and the production of physiologically active amines. Of particular concern with non-heated foods offering anaerobic conditions is the possible growth of and toxin production by *Clostridium botulinum* (Owens and Mendoza, 1985; Knochel, 1989).

Table 3 shows the intensities at which various environmental factors are singly able to prevent the growth of the main food poisoning bacteria under otherwise optimum conditions (Owens and Mendoza, 1985). It is evident that neither the high-salt nor low-salt lactic fermented fish products will support the growth of any of these bacteria once they are prepared due to their salt content and/or low pH value. However, the improper storage of raw fish before salting and insufficient acid production in very low salt fermentation can cause the outbreak of botulism. The botulinum toxin is relatively easily destroyed by cooking but very stable in salty and acidic environments (Huss and Rye Pederson, 1980). The fermented fish products most often incriminated in *C. botulinum* type E poisonings are Isushi (a type of Narezushi) and Kirrikomi (a type of Shiokara) in Japan, salmon egg cheese (fermented crushed salmon roe) among Eskimos and Indians in Canada and Alaska, Rakeorret in Scandinavia (Bartl, 1972; Sakaguchi, 1979). The Norwegian Rakeorret has caused several outbreaks in Denmark by both *Clostridium botulinum* type E and B (Knochel, 1989). Therefore, the Danish legislation decrees that commercial products with less than 6% NaCl (w/w) and pH > 5 can only be made by authorised manufacturers and all distribution and storage should be at less than 5°C.

A number of parasitic worms may be contracted by eating raw or partially cooked fish, but the relative importance of high-salt fermented or low-salt lactic fermented fish products are rarely reported. Larvae of the parasite *Anisakis* are often found in North European pelagic fish. They may cause appendicitis-like symptoms in humans and in several cases salted Matjes has been reported as a vehicle. One solution is to freeze Matjes after salting. Danish regulations demand that marinated herring should be pre-treated with brine (1:1) consisting of minimum 15% NaCl and 5% acetic acid for at least one week before being marinated in retail packs in order to kill the larvae (Knochel, 1989).

The physiologically active amines such as histamine formed by the bacterial decarboxylation of histidine, may be produced in amounts sufficient to cause poisoning in certain fishes (Eitenmiller et al., 1982). However, to what extent such amines are problems or potential problems with fermented fish is not clear (Owens and Mendoza, 1985).

Recent developments and future prospect

The need for quality improvement and process innovation in the area of fish fermentation have been widely recognised in recent years. The acceleration of enzymic hydrolysis in fish sauce making has been the major concern in the Philippines, Taiwan, Indonesia and other Southeastern countries (Mabesa and Baban, 1989; Chen, 1989; Putro, 1989). Enzymes from koji and fruits were added and in some cases selected proteolytic bacteria, for example *Brevibacterium sp.*, *Bacillus sp.* and *Micrococcus sp.*, were used for the acceleration of fish sauce fermentation. Many investigators found substantial flavour changes

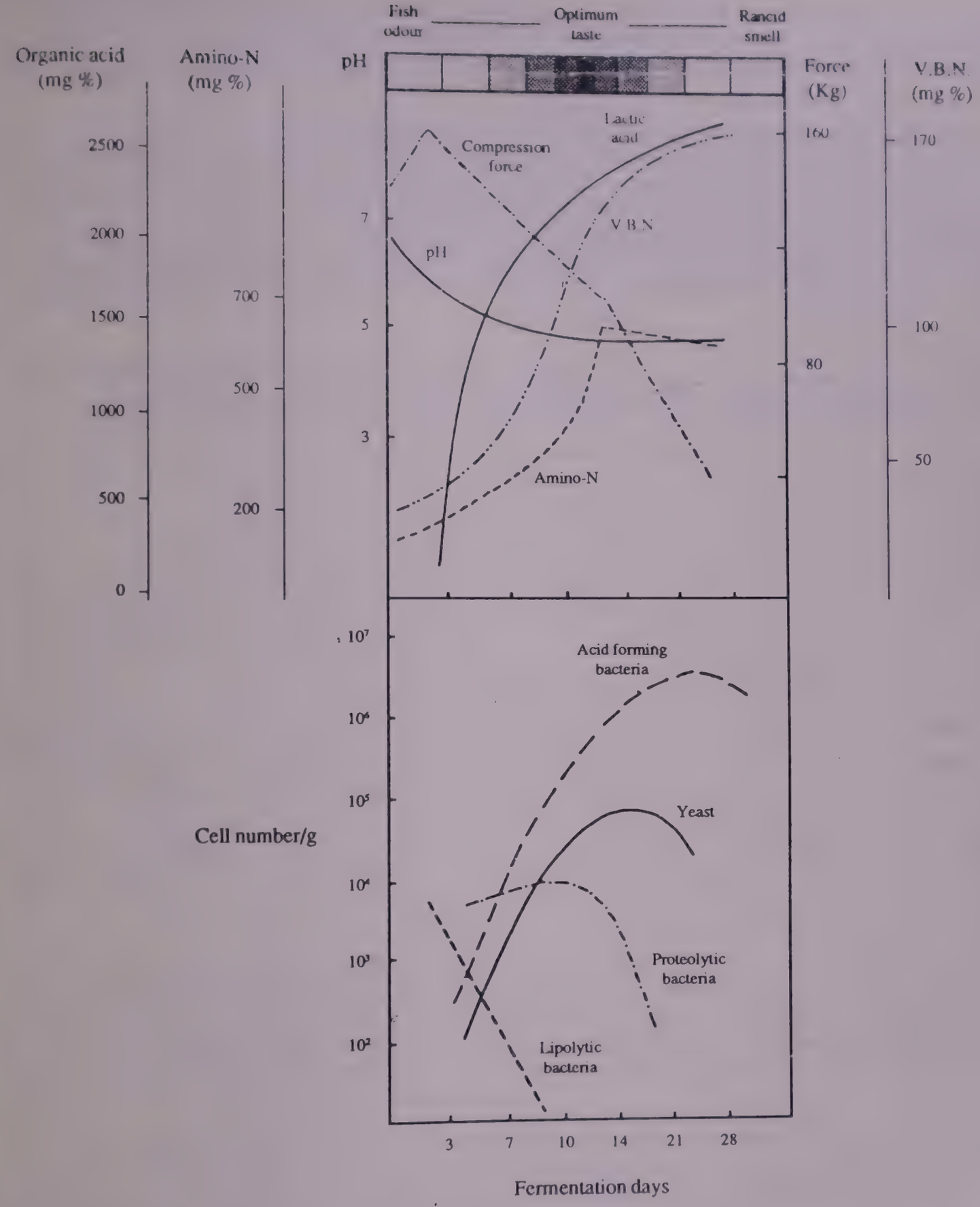

Figure 7. Microbial and biochemical changes during the fermentation of a lactic fermented fish product, Sikhae (Lee et al., 1986)

by the addition of enzymes, but the results from added microorganisms were generally satisfactory (Chen, 1989; Mheen, 1989). The traditional cottage level small scale fish sauce factories are amalgamating in many countries, and quality standardisation including proper packaging are required in most of Southeastern countries. One successful case of the industrialisation of fish sauce is Ngapi production in Burma (Tyn, 1989).

The prime concern in cured fish production is to keep the salt content as low as possible. During the scale-up of cured fish production from the traditional household level, more salt is added in order to ensure the prevention of health hazard microorganisms and quality changes during the long distribution process. However, high salt concentration generally reduces the sensory quality of the products. In addition, the strong correlation between salt intake and high blood pressure makes people doubtful now about eating salty food. Lee, E. H. (1989) has conducted an extensive study on the reduction of salt concentration of different type of Jeotkal. He could successfully preserve Jeotkal products with 8% salt incorporated with 0.5% lactic acid, 6% sorbitol and 4% of alcohol extract of red pepper. This type of product is distributed through the cold chain system and growing in popularity in Korea.

Table 3. Maximum sodium chloride concentrations and minimum temperatures, pH values and water activities for growth of food poisoning bacteria under otherwise optimal conditions (Owens and Mendoza, 1985)

Bacteria	Conditions allowing growth/toxin production			
	Minimum values			Maximum salt concentration (% w/w)
	Temp. (°C)	pH	Aw	
Bacillus cereus	7	4.4-5.0	0.93-0.95	7.5
Clostridium botulinum				
types A and B	10-12	4.8	0.94-0.95	10
type E	3.3	5.0	0.97	5
C. perfringens	15-20	5.0	0.95-0.96	4-6
Salmonella species	5.2	4.1-5.5	0.95	8
Staphylococcus aureus				
growth : aerobic	6.7	4.3	0.86	16-18
anaerobic	6.7	4.7	0.90	14-16
toxin : aerobic	10	4.3	0.90-0.93	12-13
anaerobic	10	6.5	0.90-0.93	12-13
Vibrio parahaemolyticus	3-13	4.8	0.94	8-10

A similar requirement is also imposed on lactic fermented fish products. The shelf-life of lactic fermented fish product is primarily dependent on the salt concentration, storage temperature, amount and kind of carbohydrate source and the use of other preservative materials such as garlic, tamarind, and other chemical agents. The search for natural preservative materials useful for vegetable lactic fermented products has been widely conducted in Korea (Yoon, 1987; Souane et al., 1987). The optimum processing conditions for the production of stable, low-salt and safe products by using different lactic acid producing starter cultures have been studied (Cooke et al., 1989; Lee et al., 1989).

The production of fish sauce and fish paste has faced strong competition with soybean sauce and paste and other condiments such as yeast extract, beef extract etc. However, the deep roots of traditional taste preference will keep fermented fish products the dominating condiment in the Southeastern Asian countries, as long as the products meet the requirement of today's market, such as convenience, good packaging and consistent quality. An alternative form of fish sauce in the future will be the mixture of fish sauce and soysauce or other form of condiments (Steinkraus, 1983).

For the cured fish and lactic fermented fish products in Asia, the development in the Scandinavian countries, such as pickling of salted fish associated with low temperature storage, indicate a significant future direction. The cold-chain distribution of low-salt cured fish and lactic fermented fish products is already well established in Korea and Japan. With this new development the consumption of fermented fish products is again increasing in Korea.

References

Adams, M.R. 1986. Fermented flesh foods. In: Progress in Industrial Microbiology, M.R. Adams. (ed.), 23, pp. 179-180.

Bartl, V. 1972. Semi preserved foods: general microbiology and food poisoning. In: The Microbiological Safety of Food, B.C. Hobbs and J.H.B. Christian (eds.), Academic Press, London, pp. 89-106.

Boon-Long, N. 1987. Traditional Fermented Fish Products of Thailand, Report to UN University, Tokyo.

Chang, C.H. 1976. Studies on the history of Korean traditional fish fermentation technology, Thesis Collection of Sung Sim Womens University, Seoul, Korea. 7. pp. 79-183.

Chen, H.C. 1989. Fish fermentation technology in Taiwan. In: Fish Fermentation Technology, C.H. Lee, K.H. Steinkraus and P.J.A. Reilly (eds.), YuRim Publish. Co., Seoul, pp. 185-194.

Chung, S.Y. and Lee, E.H. 1976. The taste compounds of fermented Acetes chinensis. Bull. Korean Fish. Soc., 9(2):79-110.

Cooke, R.D., Twiddy, D.R. and Reilly, P.J.A. 1989. Lactic fermentation of fish as a low-cost means of food preservation. In: Fish Fermentation Technology, C.H. Lee, K.H. Steinkraus, and P.J.A. Reilly (eds.), Yurim Publish. Co., Seoul, pp. 267-278.

Eitenmiller, R.R., Orr, J.H. and Wallis, W.W. 1982. Histamine formation in fish; microbiological and biochemical conditions. In: Chemistry and Biochemistry of Marine Food Products, R.E. Martin, G.F. Flick, C.E. Hebard and D.R. Ward (eds.), AVI. Westport, Conn., pp. 39-50.

Huss, H.H. and Rye Pedersen, E. 1980. The stability of Clostridium botulinum type E toxin in salt and low acid environment. J. Fd. Technol., 15: 619-627.

Ishige, N. 1989. Cultural aspects of fermented fish products in Asia. In: Fish Fermentation Technology, C.H. Lee, K.H. Steinkraus and P.J.A. Reilly (eds.), YuRim Publish. Co., Seoul, pp. 21-36.

Ito, H., Tachi, H. and Nikkuni, S. 1989a. Halophilic mechanism of isolated bacteria from fish sauces. In: Fish Fermentation Technology, C.H. Lee, K.H. Steinkraus and P.J.A. Reilly (eds.), YuRim Publish. Co., Seoul, pp. 229-236.

Ito, H., Tachi, H. and Kikuchi, S. 1989b. Fish fermentation technology in Japan. In: Fish Fermentation Technology, C.H. Lee, K.H. Steinkraus and P.J.A. Reilly (eds.), YuRim Publish. Co., Seoul, pp. 161-170.

Kanazawa, A. 1986. Katsuo-bushi. Seminar on traditional foods and their processing in Asia. Tokyo University of Agriculture, Nov. 13-15, Tokyo, Japan.

Karim, M.I.A. 1989. Fermented fish products in Malaysia. In: Fish Fermentation Technology, C.H. Lee, K.H. Steinkraus and P.J.A. Reilly (eds.), YuRim Publish. Co., Seoul, pp. 89-98.

Kim, Y.B., Seo, Y.G. and Lee, C.H. 1989. Growth of microorganisms in dorsal muscle of Gulbi during processing and their effects on its quality. In: Fish Fermentation Technology, C.H. Lee, K.H. Steinkraus and P.J.A. Reilly (eds.), YuRim Publish. Co., Seoul, pp. 257-266.

Knochel, S. 1989. Processing and properties of North European pickled fish products. In: Fish Fermentation Technology, C.H. Lee, K.H. Steinkraus and P.J.A. Reilly (eds.), YuRim Publish. Co., Seoul, pp. 195-210.

Lee, C.H. 1989. Fish fermentation technology in Korea. In: Fish Fermentation Technology. C.H. Lee, K.H. Steinkraus and P.J.A Reilly (eds.), YuRim Publish. Co., Seoul, pp. 171-184.

Lee, C.H., Lee, E.H., Lim, M.M., Kim, S.H., Chai, S.K., Lee, K.W. and Koh, K.H. 1987. Fermented Fish Products in Korea. YuRim Publish. Co., Seoul.

Lee, C.H., Cho, T.S., Lim, M.H., Kang, J.W., and Yang, H.C. 1983. Studies on the sikhae fermentation made by flat-fish. Korean J. Appl. Microbiol. Bioeng., 11(1):53.

Lee, C.H., Lee, E.H., Lim, M.H., Kim, K.H., Chae, S.K., Lee, K.W., and Koh, K.H. 1986. Characteristics of Korean fish fermentation technology. Korean J. Dietary Culture, 1(3):267-279.

Lee, C.H., Souane, M. and Kim, C.S. 1989. Microbiology of Gajami sikhae fermentation. In: Fish Fermentation Technology, C.H., Lee, K.H. Steinkraus, and P.J.A. Reilly (eds.), Yurim Publish. Co., Seoul, pp. 279-293.

Lee, E.H. 1989. Microbiology and biochemistry of low-salted fish fermentation. In: Fish Fermentation Technology, C.H. Lee, K.H. Steinkraus and P.J.A. Reilly (eds.), Yurim Publish. Co., Seoul, pp. 237-256.

Lee, J.G. and Choe, W.K. 1974. Studies on the variation of microflora during the fermentation of anchovy, *Engraulis japonica*. Bull. Korean Fish. Soc., 7(3):105-113.

Lee, S.W. 1989. Cultural aspects of Korean fermented marine products in East Asia. In: Fish Fermentation Technology, C.H. Lee, K.H. Steinkraus and P.J.A. Reilly (eds.), Yurim Publish. Co., Seoul, pp. 37-47.

Mabesa, M.C. and Baban, J.S. 1989. Fish fermentation technology in the Philippines. In: Fish Fermentation Technology, C.H. Lee, K.H. Steinkraus and P.J.A. Reilly (eds.), Yurim Publish. Co., Seoul, pp. 81-88.

Mackie, I.M. Hardy, R. and Hobbs, G. 1971. Fermented fish products. Rome, FAO.

'Mheen, T.I. 1989. Microbiology of salt fermented fishery products in Korea. In: Fish Fermentation Technology, C.H. Lee, K.H. Steinkraus and P.J.A. Reilly (eds.), Yurim Publish. Co., Seoul, pp. 211-226.

Owens, J.D. and Mendoza, L.S. 1985. Enzymically hydrolysed and bacterially fermented fishery products. J. Fd. Technol., 20:273-293.

Phithakpol, B. 1989. Fish fermentation technology in Thailand. In: Fish Fermentation Technology, C.H. Lee, K.H. Steinkraus and P.J.A. Reilly (eds.), Yurim Publish. Co., Seoul, pp. 143-152.

Putro, S. 1989. Fish fermentation technology in Indonesia. In: Fish Fermentation Technology, C.H. Lee, K.H. Steinkraus and P.J.A. Reilly (eds.), Yurim Publish. Co., Seoul, pp. 99-118.

Reilly, A. and Barile, L.E. 1986. Cured Fish Production in the Tropics. College of Fisheries, University of the Philippines in the Visayas and GTZ, GmbH.

Ruddle, K. 1989. The availability and supply of fish for fermentation in the Southeast Asia. In: Fish Fermentation Technology C.H. Lee, K.H. Steinkraus and P.J.A. Reilly (eds.), Yurim Publish. Co., Seoul, pp. 47-80.

Sakaguchi, S. 1979. Botulism. In: Foodborne Infections and Intoxications. H. Reiman and F.L. Bryan (eds.), 2nd edition Academic Press, New York, pp. 389-442.

Souane, M. 1987a. Fish fermentation technology in Senegal. Presented at UNU Workshop on Fish Fermentation Technology, June 22-26, Seoul, Korea.

Souane, M. 1987b. Studies on Gajami sik-hae Fellowship Training Report submitted to UNU. Tokyo, Japan.

Souane, M., Kim, Y.B. and Lee, C.H. 1987. Microbial characterisation of Gajami Sikhae fermentation. Korean J. Appl. Microbiol. Bioeng., 15(3):150-157.

Steinkraus, K.H. 1983. Handbook of Indigenous Fermented Foods, Marcel Dekker, Inc., New York, pp. 487-526.

Subasinghe, S. 1989. Fermented fishery products in Sri Lanka. In: Fish Fermentation Technology, C.H. Lee, K.H. Steinkraus and P.J.A. Reilly (eds.), Yurim Publish. Co., Seoul, pp. 153-160.

Tyn, M.T. 1989. Trends of fermented fish technology in Burma. In: Fish Fermentation Technology, C.H. Lee, K.H. Steinkraus and P.J.A. Reilly (eds.), Yurim Publish. Co., Seoul, pp. 119-142.

Wilson, C.A. 1976. Food and Drink in Britain, Penguin Books, Harmandsworth, England, pp. 24-25.

Yoon, S.I. 1987. Studies on the preservation of Kimchi. Report of Food Research Institute, Association of Korean Food Industries. Seoul.

POST-HARVEST TECHNOLOGY FOR TROPICAL FISH - A REVIEW

K. Gopakumar
Central Insitute of Fisheries Technology, Cochin-682029 India

Of the total world fish production (estimated to be around 91 million in 1988) India's share was 11% and India ranks 8th among the major fishing nations. The annual landing of marine and inland fisheries of India (1987-88) was 1.62 and 1.39 million tonnes respectively. Shrimp constitutes 12.3% of the marine landings of which 65% is made up of penaeid prawns. India's estimated potential resources is about 4.5 million tonnes. There has been considerable increase in inland production in recent years due to large scale aquaculture. Commercially important small pelagic fish constitute about 50% of the total marine landings. The landings of pelagic fish in the years 1986-87 and 1987-88 are presented in Table 1.

The pelagic fish of India includes oil sardines, mackerel, Bombay duck, anchovies, white baits, tuna, seer fish, baracudas, hilsa ilisha, bregmaceros, hemirhamphus, flying fish, carangids, catfish etc. The commercially important small pelagic fish which constitute about 50% of the total marine landings are oil sardines, mackerel, Bombay duck, anchovies and baracuda.

Utilisation

There has been considerable improvement in the pre-process handling and transport of fresh fish in India compared to sixties and seventies. There is wide spread use of ice all over the country. The government of India has set up modern fishing harbours in major landing centres in important maritime states like Kerala, Karnataka, Maharashtra and Gujarat in the West Coast and Tamilnadu, Andhra Pradesh and Orissa in the East Coast. Development of indigenous containers for storage and transport has resulted in transport of fresh fish to distant areas. This has considerably improved the consumption of fish in fresh condition.

The fish drying or curing industry in India has declined in recent years. Gujarat still continues to be a major fish drying centre. On an average about 20-25% of the fish landed in India is used for drying.

General nature of losses

Apparent losses due to dumping of food and other physical damages vary from place to place. It is maximum in Gujarat and minimum in Kerala. As a result of poor handling the fish tends to be down graded leading to economic losses for the industry. Such fish is used for reduction into fish meal which otherwise would have been used for direct consumption (Bostock, 1987). This condition predominantly exists in states like Gujarat, Andhrapradesh and Orissa.

Utilisation of pelagic fish

Oil sardine

The fishery is concentrated along the southwest coast of India. It is the major fishery of India and constitutes about 30% of the total marine landings. They are caught using boat seines, beach seines and gill nets and 60% of the total landings is contributed by country crafts (Madhavan et al., 1974). It is a fatty fish and the oil content varies depending upon the season. An oil content of upto 17% of whole fish was recorded in the months of October and November which concides with peak landing season. Because of the high oil content and flesh characteristics and feeding pattern the preservation of this fish poses several problems like belly bursting, rancidity and textural deterioration (Shenoy and Pillai, 1971). Belly bursting is a major problem during June to August when the fish feeding rate is high. Oil sardines cannot be stored in acceptable condition in ice for more than 3 days because of high oil content, mechanical damage and textural changes. Use of refrigerated seawater (RSW) system cannot improve the condition. It has been found that RSW stored sardines are comparable to iced ones only upto 2 days due to

Table 1. Estimated pelagic fish landings (in tonnes) in India (Anon., 1987)

Pelagic group	1986-87	% to the total marine landings	1987-88	% to the total marine landings	% to the total pelagic landings	
					1986-87	1987-88
Wolf herring	15,167	0.89	14,531	0.89	1.83	1.84
Oil sardine	30,442	1.78	128,877	7.87	3.67	16.30
Other sardine	81,211	4.75	79,369	4.85	9.80	10.04
Hilsa shads	4,653	0.27	3,136	0.19	0.56	0.40
Coilia spp.	24,202	1.41	18,211	1.11	2.92	2.30
Other shads	15,180	0.89	18,894	1.15	1.83	2.39
Setipinna sp.	3,182	0.19	1,428	0.09	0.38	0.18
Stolephorus sp.	65,751	3.84	48,990	2.99	7.94	6.20
Other clupeids	35,654	2.08	34,901	2.13	4.30	4.41
Thryssa sp.	29,944	1.75	29,736	1.82	3.61	3.76
Bombay duck	90,337	5.28	69,516	4.25	10.90	8.79
Half beaks & full beaks	1,986	0.12	2,264	0.14	0.24	0.29
Flying fish	1,645	0.10	1,385	0.08	0.20	0.18
Ribbon fish	97,624	5.71	80,354	4.91	11.78	10.16
Horse mackerel	9,677	0.57	9,621	0.59	1.17	1.22
Scads	52,037	3.04	14,585	0.89	6.28	1.84
Leather jackets	3,883	0.23	4,552	0.28	0.47	0.58
Other carangids	76,968	4.50	50,170	3.06	9.29	6.34
Indian mackerel	84,549	4.94	78,582	4.80	10.20	9.94
Other mackerel	475	0.03	110	0.01	0.06	0.01
S. commersoni	25,733	1.50	17,996	1.10	3.11	2.28
S. lineolatus	170	0.01	73	-	0.02	-
S. guttatus	9,488	0.55	13,254	0.81	1.15	1.68
Acanthocybium spp.	36	-	53	-	-	-
E. affinis	18,017	1.05	12,646	0.77	2.17	1.60
Auxis spp.	8,186	0.48	4,809	0.29	0.99	0.61
K. pelamis	4,033	0.24	5,524	0.34	0.49	0.70
T. tonggol	265	0.02	553	0.03	0.03	0.07
Other tunnies	4,755	0.28	3,938	0.24	0.57	0.50
Bill fish	1,318	0.08	673	0.04	0.16	0.09
Baracudas	4,512	0.26	7,143	0.44	0.54	0.90
Mullets	3,200	0.19	5,868	0.36	0.39	0.74
Unicorn cod	621	0.04	973	0.06	0.07	0.12
Miscellaneous	23,707	1.39	28,033	1.71	2.88	3.54
Total	828,599	48.44	790,768	48.30	100.00	100.00

development of rancidity. A chilled seawater (CSW) system was found slightly better than RSW. Both CSW and RSW are suitabl for storing small pelagic fish for preventing mechanical damage due to crushing usually occurring during ice storage. A majo portion of oil sardines are consumed fresh and the potential markets are within 300 km away from the landing centres. Generall the fish is packed with ice in containers like bamboo or palmirah leaf baskets and tea chests and are transported in uninsulate trucks and lorries covered with tarpaulins. Sardines caught by country crafts reaches the shore within 2 h of catch and are usuall still in rigor mortis. No ice is used in the crafts because of lack of space. They are immediately iced on shore in the ratio of 1 with finely crushed ice and reaches the destination in 4 to 6 h in good condition. At present the loss due to spoilage and physic damage is only less than 5%.

Freezing is an alternate method for prolonged preservation of the fish. Belly bursting is a serious problem encountered in medium sized sardines with low fat (Perigreen et al., 1975). A dip treatment in 15% brine for 30 min. is effective to reduce belly bursting during thawing of frozen fish. Another major problem is the development of rancidity which can be minimised by using antioxidants like, ascorbic acids, BHA, BHT and hydroquinone (Cyriac et al., 1966). The usual frozen storage temperature in India is -18 to -23°C. The shelf-life of frozen oil sardine is around 4 months. Variations are noticed in the frozen shelf-life depending on the fat content and quality of the raw material Reducing the temperature by about 10°C increases the shelf-life two-fold (Perigreen and Joseph, unpublished data). However, at present no commercial freezing of oil sardine is practised in India.

Canning of oil sardine is done in a limited scale for utilisation by the army. Abnormally high cost of containers compared to the very low cost of the fish have mitigated against the development of a lucrative canning industry. Refined vegetable oil sauces and its own juice are used as canning medium. The canning industry can become economical only if a low cost packaging material is used. Retortable pouches are being tried as an alternative to metal cans (CIFT-ODNRI project report). The technology for making retortable pouches using aluminium foil, polyester and polypropylene films is available. The studies conducted in collaboration with ODNRI, UK is expected to make much headway in developing suitable processing technique for packaging sardine in retortable pouches even though some problems like rancidity on prolonged storage and incomplete evacuation of air are noticed at present.

Mackerel

Mackerel constitutes about 10% of total marine landings. The landings are mainly by country crafts and small mechanised boats and because of the small size of the vessels on board chilling is not practised. Generally the catch reaches the shore within 2-6 h after catching and is consumed as fresh fish. The fat content varies from 3-12% depending on season and only a very small quantity is preserved by freezing, canning, curing and drying. Chilling, transportation and marketing is similar to that of oil sardines. Properly iced and stored fish remains in good condition for 6-8 days (Perigreen et al., 1975). Mackerel and seer can be stored in refrigerated seawater with good results. The major problems during frozen storage are the development of rancidity, discolouration and tough texture. It can be stored at -10°C for 6-8 weeks, at -20°C for 16-20 weeks and at -30°C for more than 40 weeks (Chinnamma and Muraleedharan, unpublished). It is noted that storing at -30°C is better than storing at -20°C after treating with antioxidants. Canning of mackerel is done either as dressed fish in tall cans or as skinless fillets in dingley cans. The filling medium used are refined vegetable oil or sauce. The metal cans used are tin cans. Easy open aluminium cans are only rarely used. Salt cured mackerel is a popular product for the domestic market. The curing is done by keeping the fish in curing tanks with salt ratio of 3:1 to 8:1 until marketed as wet cured, although small quantities are also marketed as partially or fully dried. The period of salting varies from 18 h to several days. Rust, rancidity, pink discolouration, mould growth and insect infestation are the major problems encountered in curing mackerel. Sprinkling a mixture of calcium propionate containing 0.5% butylated hydroxyanisole on fish prior to packing is found to control fungal attack, red halophiles, rancidity and rust (Valsan, 1985).

Bombay duck

It is caught by dol net mainly from the Sourashtra and Maharashtra coasts of India during November to February. It is the third largest fishery. It is not consumed fresh because of its high moisture content of 88-90% and the flesh disintegrates on cooking/frying. Marketing and consumption of the fish is in the dried form. Hanging on ropes by hooking two fish by the teeth is the common practice followed by drying in the sun. Because of the high moisture content wrinkling occurs during drying and the fish loses its appearance and is therefore not highly appreciated in sophisticated markets. They are therefore marketed among plantation workers in Southeast Asian countries. Improvements in the drying operation are possible and the fish can be processed as dried laminated Bombay duck. It is done by splitting the partially dried fish and drying further on mats to a moisture level of below 30% (Kandoran et al., 1969). It is then pressed between rollers and dried again on mats to a moisture content below 15%. The dried fish is trimmed and packed as consumer packs. Such dried laminated Bombay duck finds acceptance in overseas markets.

Anchoviella

Eighty percent of the anchoviella production is dried and the major problems are its small size and delicate nature. As a result of this the fish gets easily damaged during bulk chill storage and hence commercial icing is unsuitable. This is the major

reason for not marketing the fish in interior places in fresh condition. At present only 20% is marketed as fresh fish in the coastal areas. Traditionally the fish is dried by spreading on the beach. The final products usually contains at least 20% sand adhering to the surface. The dried fish is at present exported mainly to Sri Lanka and fetches comparatively poor returns. If the fish can be processed under hygienic conditions minimising the sand content and thereby improving the quality the product will fetch better price in export markets. The researches made in the country have recommended suitable improvements in the sun drying of the fish (Prabhu, unpublished). Blanching of fish in warm water (40°C) to eliminate slime and the adhering dirt, treatment with mild solution of alum to minimise the sticking of the fish to the drying surface, drying of the treated fish on raised platforms or mats, treatment with antioxidants to improve the colour and minimise rancidity, packing the fish in suitable containers are some of the modifications suggested to improve the quality and shelf-life. Preserving the fresh fish in a mixture of water and ice has been found to keep the fish in good condition for upto 48 h.

Spiced pickles of good quality can also be produced from anchoviella both as dry pickles and wet pickles in vinegar. Dressed fish is deep fried in vegetable oil and mixed with suitable condiments depending on the acceptability of various markets. Anchoviella and oil sardine mixed in suitable proportions and stored in salt at a ratio of 3:1 to 4:1 give patties or fish sauce a good aroma.

Ice storage shelf-life of Indian fish

Extensive investigations carried in India have shown that Indian marine fish can be kept stored in ice upto a period varying from 8 to 14 days depending on the species (Table 2). The freshwater fish have more extended ice storage shelf-life compared to cold water fish (Table 3). Bigger fish have more ice storage shelf-life compared to smaller ones and lean fish more than fatty fish.

Table 2. Ice storage shelf-life of Indian marine fish

Species		No. of days	References
Oil sardine	*Sardinella longiceps*	5-6	Surendran et al. (1988)
Mackerel	*Rastrelliger kanagurta*	6	"
Threadfin bream	*Nemipterus japonicus*	27	Govindan (1971)
Perches	*Argyrops spines*	9	Solanki et al. (1977)
Seer	*Scomberomorus gaffatus*	12-14	Perigreen et al. (1975)
Pomfret black	*Parastromateus niger*	7-9	Kurnta and Madhavan (1973) Venkataraman et al. (1967)
Pomfret silver	*Pampus argenteus*	14	Venkataraman et al. (1967)
Crabs	*Scylla serrata*	11	Chinnamma George (1984)*
	Portunus pelagicus	8	"
Mussel	*Perna viridis*	8	"
Clam (black)	*Villorita cyprinoids*	9	"

* Ph.D. (Thesis) University of Cochin (1984)
Biochemical changes associated with processing of shellfish and flavour constituents of body meat and claw meat of crab.

Table 3. Ice storage shelf-life of Indian freshwater fish

Species	No. of days	References
Pearlspot (*Etroplus suratensis*)	12-14	Surendran et al. (1988)
Milkfish (*Chanos chanos*)	14	"
Tilapia (*Oreochromis mosambica*)	12-13	"
Mullet (*Liza corsula*)	8	Varma et al. (1983)
Carps (all species)	16-21	Lahiry et al. (1963)
Catfish (*Wallagu atu*)	16-21	"
Carps (*Catla catla*)	18	Bandopadhyaya et al. (1986)
Labeo fabriatus	18	"
Labeo rohita	10-12	Bandopadhyaya et al. (1985)
Labeo calbasu	8-17	"
Labeo gonius	16-23	
Cirrhinus mrigala	13-17	
Cirrhinus reba	12	

Histamine content of Indian fish

Histamine, the toxic degradation product of the amino acid histidine presents a serious health problem to food scientists all over the world. Large amounts of histamine are often detected in highly spoiled fish, particularly *Scombroid* species and some other pelagic fish. Most pelagic fish examined recently at Central Institute of Fisheries Technology, Cochin, were found to contain varying population of histidine decarboxylating bacteria ranging from 40 cfu/g in seer fish to 1.12 x 10^4 cfu/g in Malabar herring (Ames et al., 1987). All fish species examined were in prime condition of freshness. No direct correlation was observed between the histamine content in the fish and the population of histidine decarboxylating bacteria. The results are presented in Table 4.

Production of histamine in oil sardine (*Sardinella longiceps*) and mackerel (*Rastrelliger kanagurta*)

Oil sardine and mackerel are perhaps the most important pelagic fish consumed fresh in large quantities in India. Extensive investigations carried out at CIFT showed that no significant amounts of histamine are formed in oil sardine if stored at 0°C. At 10°C the amount of histamine produced remains low (0-3.1 mg%). However, at 23°C after 20 h several fish samples developed histamine at levels greater than 20 mg% (toxic limit), even though the fish remained highly acceptable by sensory standards. After 25 h at 23°C the fish showed histamine content varying from 10 to 155 mg% and became unacceptable. After 29 h at 23°C the quality of the fish was very bad and some contained histamine as high as 174 mg%. At ambient temperature (30+/-3°C) the fish became unacceptable after 15 h and developed histamine as high as 233 mg%. One interesting aspect of the study of the market samples was that the histamine content varied from batch to batch and within each batch. In both cases the fish were seen in prime condition of freshness by visual and sensory standards.

Table 4. Histamine content of individual fish in relation to their content of histidine decarboxylating bact

Fish		Histamine content mg/100g of fish				Histidine decarboxylati bacteria (cfu/
		1	2	3	4	
Jew fish	*Otolithus spp.*	0.15	0.06	0.15	0.41	$3.3x10^3$
Indian mackerel	*Rastrelliger kanagurta*	0.20	0.14	0.42	0.29	$2.2x10^2$
Carangida	*Chorinemus spp.*	0.88	0.25	0.81	1.30	$4.4x10^2$
Malabar herring	*Thrissocles spp.*	0.49	0.18	0.00	0.00	$1.1x10^4$
Silver belly	*Leognathus spp.*	0.30	0.20	0.00	0.00	$6.4x10^3$
Kilimeen	*Nemipterus japonicus*	0.18	0.16	0.00	0.00	$2.0x10^2$
Seer fish	*Scomberomorus spp.*	0.53	0.38	0.00	0.00	$0.4x10^2$
Carangid	*Thiriyan* (local name)	0.00	0.00	0.00	0.00	$0.9x10^2$
Lactarius	*Lactarius lactarius*	0.00	0.00	0.00	0.00	$3.9x10^3$
Ribbon fish	*Trichiurus spp.*	0.00	0.00	0.00	0.00	$2.0x10^3$

* cfu = colony forming units

Extensive ice storage studies were conducted on Indian mackerel on board the research vessel FORV Sagar Sampada samples were immediately iced and others kept stored at 10°C (ice + water) and ambient temperature 26°C. It was seen 0 to 2°C even after 72 h of storage fish developed only very low levels of histamine (mean value for 4 samples 0.41 mg%) fish was rated fair by sensory tests. At 10°C after 72 h the levels of histamine was 13.4 mg% (mean for 4 samples) and at after 32 h showed 260 mg% (mean for 4 samples). However, it should be noted that at 10°C after 556 h and at 26°C afte the fish were inedible.

The study conclusively proves that sardine and mackerel will not develop histamine if kept stored below 10°C.

Microbiology of cultured freshwater fish

The bacteriological changes of the skin surface, gills and intestines of rohu, mrigal, calbasu, catla and milkfish are in Table 5. The total bacterial populations of these fish were more or less similar, the lowest bacterial counts being on th surface and the highest for intestine with contents. The standard plate count (SPC) at 37°C was 50 to 80% of the SPC at temperature (28+/-2°C) in the case of freshwater cultured fish and only about 10% in the case of brackishwater cultured indicating that the freshwater cultured fish harboured more mesophiles, compared with brackishwater cultured fish. Also, cu species were found to harbour significant population of coliforms, including *Escherichia coli* and *Faecal streptococci*, indi that the aquatic systems where the fish were cultivated had been contaminated with bacteria of faecal origin.

These fish, when stored in ice remained in acceptable condition upto 17-18 days. An interesting observation was tha when the SPC of the muscle (with skin) had crossed the one million mark, the fish were organoleptically quite acceptable observation is quite different from those of the tropical marine fish, in which case, the fish became quite unacceptable by 6- in ice, at which time their SPC were above the one million mark.

Table 5. Total bacterial counts of skin surface, gills and intestine with contents

	Room Temp.	37 °C	Coliforms (MPN)	*E. coli* (MPN)	Coagulase +ve *Staphylococci*	*Faecal streptoccoci*
Rohu						
Skin surface/cm²	$2.1x10^4$	$1.0x10^4$	33	nil	34	66
Gills cfu/g	$6.8x10^4$	$2.3x10^4$	118	14	nil	128
Intestine with contents cfu/g	$7.5x10^5$	$1.2x10^4$	640	82	nil	156
Mrigal						
Skin surface/cm²	$1.6x10^4$	$5.8x10^3$	418	28	nil	nil
Gills cfu/g	$1.5x10^5$	$4.8x10^4$	5,100	14	nil	42
Intestine with contents cfu/g	$5.5x10^4$	$4.2x10^4$	27	nil	nil	nil
Calbasu						
Skin surface/cm²	$3.8x10^3$	$1.0x10^4$	284	11	-	42
Gills cfu/g	$6.3x10^4$	$2.7x10^4$	814	62	-	118
Intestine with contents cfu/g	$2.8x10^5$	$8.2x10^4$	1,200	118	-	640
Catla						
Skin surface/cm²	$7.2x10^3$	$4.1x10^3$	5	nil	nil	28
Gills cfu/g	$1.6x10^5$	$9.4x10^4$	300	62	nil	150
Intestine with contents cfu/g	$8.3x10^6$	$9.2x10^6$	6,200	246	nil	218
Milkfish						
Skin surface/cm²	$6.1x10^4$	$1.4x10^3$	110	-	124	168
Gills cfu/g	$2.4x10^5$	$1.7x10^4$	242	18	42	212
Intestine with contents cfu/g	$7.9x10^5$	$2.0x10^4$	634	62	-	464

This difference in shelf-life of cultured fish from that of the marine fish amply points to the fact that there are bound to be difference in the types and biochemical activities of the bacteria of cultured freshwater and brackishwater fish.

Changes in the bacterial flora of rohu, mrigal, calbasu, catla and milkfish held in ice storage are presented in Table 6. The microflora of these fish comprised of Gram positive bacteria, mainly *Micrococcus* and *Bacillus*. Other Gram positives like *Arthrobacter*, *Lactobacillus*, *Staphylococcus* and *Streptococcus* were also present. Other bacterial species found were *Pseudomonas*, *Acinetobacter* and *Enterobacteriaceae*.

During iced storage of these fish, there was the establishment of a predominantly *Pseudomonas* group, just like in the marine fish. However, even though *Pseudomonas* group attained a dominant position, at the time the fish became unacceptable organoleptically, there was no spoilage or foul smell usually observed in the case of the spoiling marine fish. This naturally indicated that the role of bacteria in the spoilage of cultured fresh and brackishwater fish is very limited.

Table 6. Changes in the major bacterial flora of freshwater fish during ice storage

Fish	Bacterial flora	No. of days of ice storage			
Catla		0	7	11	20
	Pseudomonas	8	16	50	62
	Moraxella	12	6	-	-
	Acinetobacter	20	26	12	6
	Vibrios	16	12	-	-
	Micrococcus	20	16	32	28
Mrigal		0	6	14	22
	Pseudomonas	20	14	26	56
	Acinetobacter	20	52	40	12
	Micrococcus	30	28	18	16
	Bacillus	24	2	-	-
Calbasu		0	5	12	20
	Pseudomonas	12	8	24	65
	Acinetobacter	24	32	28	14
	Arthrobacter	10	10	6	2
	Micrococcus	32	36	30	16
Rohu		0	6	12	20
	Pseudomonas	20	14	20	70
	Acinetobacter	10	56	10	4
	Arthrobacter	-	-	10	10
	Micrococcus	60	28	40	10
Milkfish		0	6	-	25
	Pseudomonas	20	23	-	68
	Acinetobacter	12	17	-	8
	Moraxella	15	14	-	7
	Vibrios	16	10	-	2
	Micrococcus	10	8	-	2
	Alcaligenes	8	8	-	5
	Bacillus	8	3	-	1

References

Ames, G.R., Gopakumar, K. and Vijayan, P.K. 1987. Infofish, 2/87, 41.

Anon. 1987. Statistics of Marine Products exports, Marine Products Export Development Authority, Cochin.

Bandopadhyaya, J.K., Chattopadhyay, A.K. and Battacharya, S.K. 1986. Fish. Technol., 23, p.140.

Bandopadhyaya, J.K., Chattopadhyay, A.K. and Battacharya, S.K. 1985. In: Proceedings of the Symposium on Harvest and Post-Harvest Technology of Fish, Society of Fisheries Technologists, India, p. 381.

Bostock, T. 1987. Marine fishers of Gujarat: Post-harvest losses and possibilities for development. TDRI Report L75, ODNRI, London.

Cyriac, M., Choudhury, D.R. and Pillai, V.K. 1966. Fish. Technol., 3, 30.

Govindan, T.K. 1971. Indian Fd. Packer, 25, 27-32.

Kandoran, M.K., Solanki, K.K. and Venkitaraman, R. 1969. Fish. Technol., 6, 140.

Kumta, U.S. and Madhavan, V.N. 1973. Acta Aliment., 2, 467.

Lahiry, N.L., Moorjani, M.N. and Baliga, B.R. 1963. Fd. Technol., 17, 1203.

Madhavan, P., Unnikrishnan Nair, T.S. and Balachandran, K.K. 1974. Fish. Technol., 11, 8.

Perigreen, P.A., Surendran, P.K., Govindan, T.K. and Ayyappan Pillai, S. 1975. Fish. Technol., 12, 105.

Shenoy, A.V. and Pillai, V.K. 1971. Fish. Technol., 8, 37.

Surrendran, P.K., Joseph, J., Shenoy, A.V., Perigreen, P.A., Iyer, K.M. and Gopakumar, K. 1988. Fisheries Research, 7, 1- 9.

Valson, A.P. 1985. Harvest and Post-Harvest Technology of Fish, Society of Fisheries Technologists, India, p.566.

Varma, P.R.G., Mathen, C. and Francis, T. 1983. J. Fd. Sci. Technol., 20, Sept.-Oct., 219.

Venkataraman, R., Prabhu, P.V. and Mankad, D.J.A. 1967. Indo-Pacific Council Occasional Paper, FAO, 167, 8.

Criteria of Fish Spoilage

Keith H. Steinkraus
Institute of Food Science, Cornell University, Ithaca, New York 14853 USA

Summary

Fresh fish are very susceptible to spoilage; and, therefore, nearly all fish products undergo at minimum some staling or incipient spoilage. Fish are considered to be spoiled when the flavour/aroma/colour or texture has been modified beyond limits acceptable to the consumer. Enzymes in the fish tissues start autolysis reactions as soon as the fish dies. Enzymes from bacteria present in or on the fish contribute to deteriorative changes in the fish if allowed to multiply. While a trained sensory evaluation panel may be the genuine test of incipient spoilage, there are a number of analytical methods that can be used to measure/quantitate early evidence of deterioration. Among these are (1) Total bacterial count (TBC) particularly of psychrophiles and mesophiles; (2) Free glucose which is low in fish tissue and disappears rapidly if bacteria are growing; (3) Inosine monophosphate (IMP) which is a flavour enhancer; as it disappears the sensory score tends to decrease; (4) Hypoxanthine increases as IMP decreases so it is a useful measure of deterioration. It accumulates and reaches a maximum after 2 weeks storage; (5) Bound ribose decreases as hypoxanthine increases; (6) Volatile acids tend to increase as bacterial count increases; (7) Trimethylamine increases with storage and reflects a decrease in sensory quality. The above and other methods of measuring fish spoilage will be discussed.

Introduction

The normal pathway of all animal and plant life at time of death is recycling back to soil, a process which can be described as spoilage. This is accomplished primarily by microorganisms which have had this task since the beginning of the earth 3.5 to 4.5 billion years ago. In order to obtain food and particularly to preserve it until it can be eaten, man must interfere with spoilage processes. This is accomplished through salting, dehydration, smoking, fermentation in the developing world and through, in addition to the 4 processes mentioned, canning or freezing in the developed world.

Fish and other marine products are among the world's most perishable products. In addition to microbial spoilage, tissue hydrolysis in fish occurs through autolytic enzymes in the cells and in the gut. The predominant food for fish is smaller fish and, in order to digest the fish, the gut has to be rich in proteolytic enzymes which, as soon as the fish dies, continue to liquefy, in this case, the gut and surrounding tissues of the fish themselves. This combined with microbial invasion can lead to very rapid breakdown and spoilage of the fish. These reactions can be slowed by salting, dehydration, cooling/icing, freezing, canning or smoking or they can be utilised as a desirable step in fermentation.

Since spoilage and fermentation involve some comparable chemical/biochemical changes, it is necessary to define the two terms. Spoilage is microbial and/or enzymatic hydrolysis in which flavours or aromas undesirable, unattractive or possibly revolting develop or in which toxins develop in the fish. Fermentation of fish is a process involving enzymatic hydrolysis with or without active participation of microbes in which the products are flavours, aromas and texture desirable to human consumers and containing no obvious toxic compounds. In fermentation, proteolysis is controlled, principle products are amino acids and peptides which provide a meat-like flavour to the products such as fish sauces and pastes. In spoilage, putrefaction with production of evil smelling, potentially toxic and toxic compounds are produced.

There is, however, a very delicate balance between fish spoilage and fish fermentation. Before obvious spoilage occurs, there are states of incipient spoilage. How can spoilage and in particular incipient spoilage be recognised?

Usual criteria for spoilage do not always hold true for fermented fish/shrimp. For example: Vietnamese fish sauce nuoc-mam should have a pH of about 6.0-6.4. A pH above 6.5 indicates poor keeping quality. Spoilage definitely sets in when the pH rises to 6.8 or higher and the contents of volatile nitrogen bases (VNB) ammonia and mono-di- and trimethylamines rise and are liberated (Van Veen, 1965).

The difficulty of applying usual organoleptic characters to wholesome fish paste is decribed by Van Veen (1965). Indonesian fish paste "trassi ikan" begins with small fish mixed with salt aboard fishing boats. By the time the fishing boats reach shore, the salted mass has a very disagreeable smell. The mass is dried in thin layers in the sun as soon as possible, mixed with more salt and pounded until the paste does not lose any more moisture in the sun. During processing the product may have a very bad odour and may be infested with fly maggots but the odours of decomposition gradually disappear. These strong smelling products have a very good keeping quality and maybe mixed with Spanish peppers etc. to yield very spicy products called "sambal" used regularly in Indonesia with every rice meal along with dried and salted fish.

It is very interesting to the author that, in the tropics where spoilage reactions are accelerated and the consumers of fresh fish often will buy them only if they are alive, fermented fish probably generally involve some spoilage reactions for their flavour/aroma.

Raw fish which probably requires the most careful handling and processing to prevent incipient spoilage is very popular from Japan to Thailand.

The best Vietnamese nuoc-mam has a rather strong, cheese-like odour and a salty taste. Nuoc-mam is essential to the Vietnamese consumer. The Vietnamese prefer higher quality but will accept about any quality sauce rather than eat meals without it.

In the most primitive methods, small fish are first kneaded and pressed by hand, salted and placed in earthenware pots, tightly sealed, and buried in the ground for several months. When opened the liquid portion is nuoc-mam. It is proteolytic enzymes in the fish guts that solubilise the fish tissues. Microbes in the presence of high salt concentration (above 20% w/w) have little influence, tend to die rather quickly, and, if they contribute to the flavour/aroma do so as halophiles in the later stages of fermentation.

Fish spoil rapidly. This is due, in part, to the fact that fish tissue becomes less acid post-mortem than tissues of warm blooded animals; and fish contain trimethylamine oxide (TMAO) which stimulates anaerobic growth of spoilage bacteria (Raa, 1981). The post-mortem pH of cod muscle is usually about 6.2 to 6.5.

TMAO can be used by spoilage bacteria as an electron acceptor or oxidising agent when oxygen is depleted. TMAO used in anaerobic fermentations yields more energy than strict anaerobiosis. Spoilage bacteria also can utilise lactic acid as a sole source of energy if TMAO is present. During anaerobic degradation of lactic acid, TMAO is reduced to trimethylamine (TMA), acetate and CO_2. The TMA has a characteristic spoiled fish odour.

Fish also spoil readily because they contain psychrophilic (cold loving) bacteria that grow readily even if fish is iced (Raa 1981).

If fish are in water and oxygen is therefore very limited, free glucose (also very limited in fish) is first oxidised by microorganisms using residual oxygen. Then lactic acid is metabolised as long as TMAO is available. Then anaerobic bacteria gain energy by decomposing amino acids. This yields ammonia rapidly, one molecule of acid is oxidised and one molecule of amino acid is reduced with both releasing free ammonia (the Strickland reaction).

During amino acid decomposition H_2S and ptomaines both offensive odours are produced (Raa, 1981).

In air, bacteria on the surface, decompose amino acids yielding NH_3, H_2S, mercaptans, dimethyl sulfide and those compounds along with TMA (resulting from anaerobic reactions in the deeper tissues) spoil the product rendering it unfit for human consumption (Raa, 1981).

The reactions described above can be inhibited by freezing, icing, salting, drying, smoking or combinations of these procedures.

The most obvious criterion of spoilage for the potential consumer is the presence of offensive aromas in fresh fish. In dried fish, the typical odour of oxidative rancidity may be an indication of a type of spoilage for some consumers but not all as some cultures are used to rancid dried fish that the consumers actually prefer rancidity to non-rancid products.

Some fish components and the spoilage compounds derived from them are listed in Table 1. Certain of them such as ammonia, hydrogen sulfide, dimethyl sulfide and the mercaptans are readily detected by the human nose (Table 2). And, of course, they can also be determined quantitatively by chemical and gas or HPLC chromatography methods (Liston, 1982). Total volatile bases have been suggested as a measure of spoilage; but as Jacober and Rand (1982) have shown (Table 3), spoilage can be quite advanced before TVB shows an appreciable increase. On the other hand, hypoxanthine shows a remarkable increase as cold storage continues and therefore it can serve as a good method for following deteriorative changes. Jacober and Rand (1982) suggested standards of hypoxanthine and diamines, along with analysis for spoilage compounds as a means of grading the quality of fish (Table 4).

Table 1. Some fish components and their spoilage compounds*

Substrate	Spoilage compound(s)
Urea Amino acid	ammonia
Inosine	hypoxanthine
Trimethylamine oxide	trimethylamine
Methionine Cysteine	hydrogen sulfide dimethyl sulfide methyl mercaptan
Carbohydrates, lactate	acetic acid, CO_2, H_2O
Glycine, leucine, serine	esters of acetic, propionic, butyric and hexanoic acid

* Adapted from Shewan (1977)

Table 2. Detectable and actual concentrations of certain volatiles in spoiling fish*

Compound	Detectable level (ppb)	Concentration in spoiling fish (ppb)
Hydrogen sulfide	40	150
Dimethyl sulfide	0.50	20
Methyl sulfide	0.50	120

* Adapted from Shewan (1977)

Table 3. Total volatile bases (TVB) and hypoxanthine development in winter flounder stored at 4-6°C*

Storage time (days)	TVB (mg% N)	Hypoxanthine (mg%)
0	11	5
2	11	27
4	10	68
7	13	60
9	13	89
11	18	55
14	24	65

* Adapted from Jacober and Rand (1982)

Table 4. Proposed standards for the evaluation of fish*

Grade	Hypoxanthine (μM/g)	Diamine[1]	Spoilage compounds[2]
A	<2.5	<1.5	<6.0
B	>2.5	<1.5	<6.0
C	>2.5	>1.5	>6.0
Spoiled	<2.5	>1.5	>6.0

[1] measured by diamine oxidase method
[2] measured by pyridoxal L-5'-phosphate method
* Adapted from Jacober and Rand (1982)

Although autolytic enzymic changes are likely to be the first changes in fresh, whole fish, there is some question whether or not these constitute spoilage but they may damage the texture and appearance of the fish. Autolysis does release amino acids and peptides that stimulate bacterial growth which will definitely lead to spoilage. Thus, if laboratory facilities permit, determination of psychrophilic and mesophilic bacteria on the skin may reveal the very earliest spoilage reactions. Glucose is limited in fresh fish tissue and its disappearance is an early indication that bacterial growth is occuring. Inosine monophosphate is a flavour enhancer and its disappearance is an indication that deteriorative changes are underway. Since hypoxanthine increases as inosine monophosphate disappears, it can be a very useful measure, and perhaps the most useful measure, of incipient spoilage. Measurement of total volatile acids such as acetic can be a useful indirect method of measuring microbial growth. Determination of trimethyl amine can be useful measure of fish staling and incipient spoilage. It requires a rather sophisticated laboratory. Hebard et al. (1982) have discussed in detail the possible use of TMA as an indicator of spoilage.

Readers interested in detailed methods of analysis for chemical components related to fish spoilage are referred to the excellent paper of Johns and Rand (1977).

References

Hebard, C.E., Flick, G.J. and Martin R.E. 1982. Occurrences and significance of trimethylamine oxide and its derivatives in fish and shellfish. In: Chemistry and Biochemistry of Marine Food Products, R.E. Martin, G.J. Flick and R.R. Ward (eds.).

Jacober, L.F. and Rand, A.G., Jr. 1982. Biochemical evaluation of seafood. In: Chemistry and Biochemistry of Marine Food Products. R.E. Martin, G.J. Flick and R.R. Ward (eds).

Johns, F.D., Rand, A.G. Jr. 1977. Enzyme methods to assess marine food quality. In: Enzyme in Food and Beverage Processing. R.L. Ory and A.J. St. Angelo (eds.). ACS Symposium Series 47, American Chemical Society.

Liston, J. 1982. Recent advances in the chemistry of iced fish spoilage. In: Chemistry and Biochemistry of Marine Food Products. R.E. Martin, G.J. Flick and D.R. Ward (eds.)

Raa, J. 1981. Biochemistry of microbial fish spoilage and preservation by lactic acid bacteria and added acid. In: Global Impacts of Applied Microbiology, S.O. Emejuaiwa, O. Ogunbi and S.O. Sann (eds.), Academic Press.

Shewan, J.M. 1977. The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. In: Handling, Processing and Marketing of Tropical Fish, Tropical Products Institute, pp. 51-66.

Van Veen, A.G. 1965. Fermented and dried seafood products in Southeast Asia. In: Fish as Food III, G. Borgstrom (ed.), Academic Press.

A STUDY ON THE USE OF DRY ICE IN FISH HANDLING

*Sumpeno Putro**, *R. Moeljanto***, *Jamal Basmal***, *Mei Dwi Erlina*** *and Sugiyono***
* Technical Advisor, Project RAS/84/027, INFOFISH Kuala Lumpur, Malaysia
** Research Institute of Fishery Technology (RIFT), Jalan K.S. Tubun, P.O. Box 30/Palmerah, Jakarta, Indonesia

Summary

Ice is widely used as a cooling medium for fish, mainly because it has a very large cooling effect for a given weight or volume and it is harmless, portable and cheap. The use of ice also permits rapid cooling through intimate contact between fish and ice, so that the fish are kept moist, cold and glossy and prevented from drying out. However, the amount of ice needed for chilling of fresh fish is quite substantial especially in tropical climates. A number of studies have shown that the practical rule of 50% fish and 50% ice is sometimes not applicable due to excessive loss of ice during transport, resulting in high ice costs.

The study conducted shows that with the incorporation of dry ice the ratio of ice to fish could be changed from 1:1 to 1:4. The incorporation of dry ice could also improve the efficiency of ice used for chilling of fresh fish particularly for bulk handling. With 2 kg of dry ice, the cooling capacity of ice can be extended up to 32 h, hence the fish can be transported a longer distance. One of the most interesting results of the study is the use of dry ice in live fish handling. Dry ice was found to enhance the anaesthetic effect of ice water during the immobilisation process while also maintaining the sawdust temperature of 10-12°C which otherwise tends to fluctuate considerably during transport. Common carp transported using chilled sawdust at a ratio of 10:2:2 of sawdust:ice:dry ice could be kept alive for 5 h.

Introduction

Dry ice is the solid or liquid form of carbon dioxide gas, commonly known as "fizz" or "bubbles" in soft drinks. It has an extraordinarily low temperature of -79°C, as compared to 0°C for ordinary ice. On evaporation, dry ice reverts directly into carbon dioxide, leaving no liquid or residue whatsoever. As a refrigerant, dry ice is able to remove three times the quantity of heat compared to the same volume of ice.

Dry ice is currently used as a refrigerant in ice boxes, car fridges, game bags or any kind of insulated containers. It is particularly long-lasting when the containers are stored in a cool place, away from direct sunlight, when food and drinks are pre-chilled and when containers are not opened frequently.

Although dry ice has no harmful effects on foodstuffs, food will be partially frozen if placed too near or in direct contact with it which could impair their palatability and texture. Apart from economic assessments, various technical problems associated with the use of dry ice in fresh and live fish handling remain to be solved.

Scientific background

Fish is highly perishable by nature, undergoing rapid spoilage especially under tropical conditions of high temperature and relative humidity. Spoilage starts immediately after the fish dies as a result of a series of complicated bacterial and autolytic enzyme reactions (Putro, 1989). In addition to cleanliness and careful handling, cooling of the fish immediately after catch and keeping them chilled throughout the processing and distribution process is considered absolutely necessary to prevent fish going bad too quickly. By reducing temperature, the rate at which bacteria grow and the incidence of chemical changes that contribute to spoilage of fish are kept in check.

Initial product temperature is of critical importance for prolonged frozen and chilled transport of fish and fishery products. Temperature has a pronounced effect on the growth of bacteria. If finfish fillets have a starting population of 10,000 bacteria per

gram, 8.3 days of shelf-life can be expected if they are held at 0°C. If they are held at 5°C however, the expected shelf-life is
2.6 days, and at 10°C only 1.4 days. It is recommended that the initial temperature of fresh seafood to be air-freighted shoul
about 0°C (Barnett, 1989). Experiments conducted for this study indicate that a 10-pound box of fish takes approximately
to cool from 30°C to near 0°C, whereas cooling in bags requires approximately 15 h.

The amount of ice needed for chilling of fresh fish is economically important especially in tropical countries.
introduction of new fish handling methods has not always been successful, very often due to the resultant high costs of ice. Besi
the practical rule of using 50% ice:50% fish usually cannot be applied to tropical countries (Boeri et al., 1985).

Currently, ice is widely used as a cooling medium for fish, mainly because it has a very large cooling effect for a g
weight or volume. The use of ice also permits rapid cooling through intimate contact between fish and ice. To date, neither s
nor liquid dry ice is widely used for chilling fish mainly because the temperature difference between dry ice and fish is very la
and fish in close contact with dry ice can become partially frozen. In addition, the cost of dry ice is much higher than ice ha
the same cooling capacity (Putro, 1989).

Previous studies have shown that frozen seafoods could be transported in insulated vans up to 600 km with 12-14%
ice and their good condition maintained for about 45 h (Dholakia et al., 1982).

Considering the underutilisation of substantial amounts of dry ice generated by the country's fertiliser industry, ef
to optimise its usage will be highly desirable.

The objective of the study was to explore the possible use of dry ice in fresh and live fish handling, because of the r
limited application of the country's enormous dry ice production in the food industry.

Materials and methods

Fish used in this study included: milkfish (*Chanos chanos*), skipjack (*Katsuwonus pelamis*) and brackishwater sh
(*Penaeus sp*). Common carp (*Cyprinus carpio*) were used for both fresh and live handling studies.

Preliminary trials were carried out to find the most appropriate techniques for dry ice incorporation into chilled
handling, by monitoring the temperature profiles of the fish using a thermo-recorder.

Trials

Unless otherwise stated, fish were chilled and transported in insulated wooden boxes. The fish were packed in alte
layers of ice, and a dry ice pack placed on top of the inner box, as shown in Figure 1.

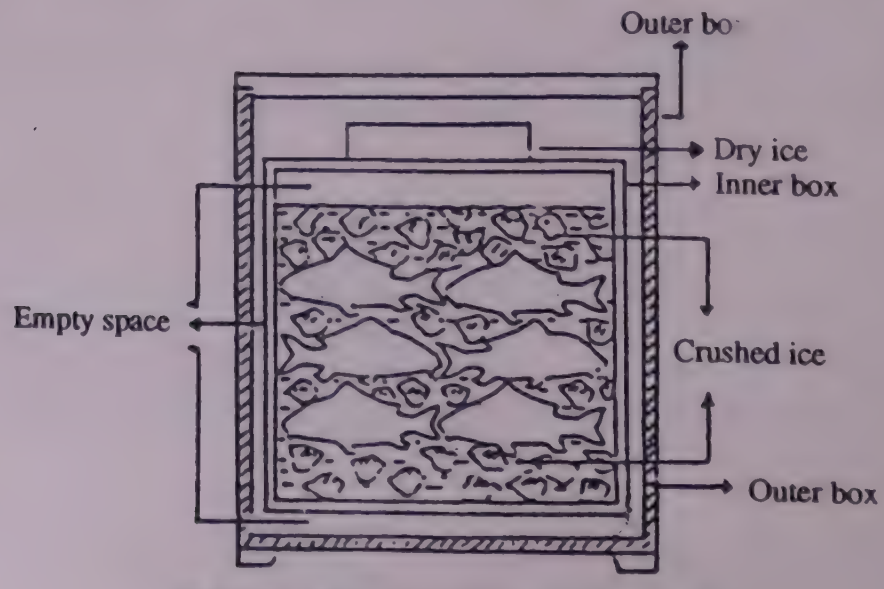

Figure 1. Chilled transport using cool box

Transportation of fish

Transport time of fresh milkfish was approximately 18 h, and the ratios of fish:ice:dry ice were 4:1:1/3; 4:1:1/5 and 4:1:1/10. The cool box capacity was 40-45 kg.

Skipjack (*Katsuwonus pelamus*) used in the study was caught by gill net in Pelabuhan Ratu waters, south coast of W. Jawa. The fish had been lying on board the fishing vessel without ice for about 3-4 h. Organoleptic evaluation showed, however, that skipjack were still at pre-rigor state. The average size of the fish was between 45-60 cm. The fish:ice:dry ice ratios used were 4:1:1/10 and 4:1:1/5.

Transport time of fresh shrimp was about 10 h. The same fish:ice:dry ice ratio was used in this study.

Newly harvested common carp with an average weight of 250 g were used in this trial. The same fish:ice:dry ice ratio was applied, and transport time was 24.5 h.

Transport of live common carp was carried out without water using a chilled sawdust medium. The sawdust was chilled to 10-12°C by leaving the sawdust overnight in a chill room. Crushed ice was slowly added into clean, freshwater until the temperature dropped to 10-12°C. The live common carp were then carefully introduced into the chilled water until anaesthetised. About 300 g of dry ice was added to speed up the process. The anaesthetised fish were then carefully wrapped with paper to prevent the gills from being clogged with sawdust before being packed in chilled sawdust. At certain time intervals, samples were taken and quickly put into running/aerated freshwater.

Transport of fresh fish in bamboo baskets was carried out to simulate widely practised fresh fish handling/transportation. In this trial, the bamboo baskets were lined with five layers of banana leaf and a layer of plastic film (Figure 2). The fish were packed in a similar manner when using a cool box. Approximately 48 kg of fish were used, and the amount of ice and dry ice added was 12 kg and 4 kg, respectively.

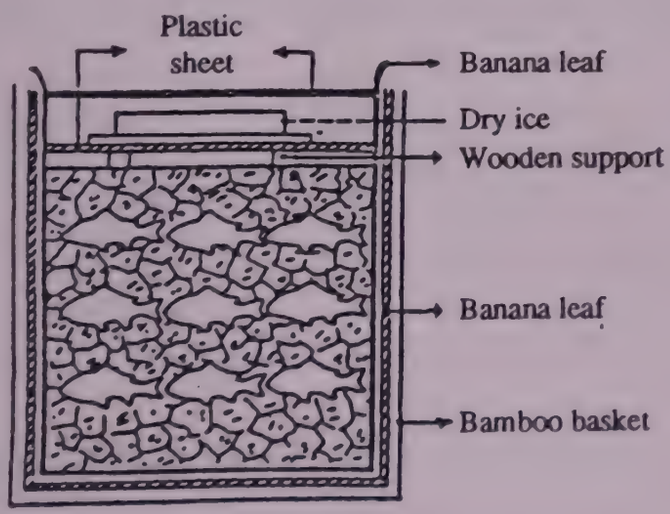

Figure 2. Chilled transport using bamboo basket

Results and discussion

Chilling

Results show that the incorporation of dry ice helps to maintain the chill temperature of milkfish as evident from the low temperature of less than 10°C in the centre of the fish body muscle as compared to 18°C control during 22 h transportation trials. Outer temperature as high as 29°C followed by rapid deterioration process were noticed for control samples. This was reflected

in the higher TVB content of 16.5 mg N for control samples as compared to 13.5 mg N for samples with 4:1:0.2 (fish:ice:dry ice) ratio. Chilled milkfish with dry ice added were still in prime quality, whereas samples without dry ice already showed significant quality deterioration upon arrival.

Temperature and quality changes of skipjack during chilled handling/transportation showed that the use of 4:1:0.2 (fish:ice:dry ice) led to considerably constant low temperatures especially in the bottom part of the container which otherwise varies between 2-10.3°C during 26 h transportation trials. Unlike the smaller (250 g) milkfish with a relatively thin body shape (3-4 cm), skipjack used in this study were bigger (3.5-5.0 kg) and had thicker body shapes (20 cm). Consequently, they displayed distinct temperature profiles during chilling, especially pertaining to the time lapse for chilled temperature penetration into the centre of the muscle. It was seen that it takes less than 4 h for milkfish to achieve a centre temperature of 4-6°C, as compared to 18.5 h for skipjack to reach 10.4°C. There was also evidence that the use of ice for chilling skipjack was only effective for the first 5 h where the surface temperature of the fish dropped to 4.5°C, followed by a rapid increase in all of the fish layers. The lowest temperature of the centre of the fish muscle was 16°C after 10 h of chilling, and gradually increased to 20°C a few hours later. The incorporation of dry ice during chilled transport (4:1:0.2) had a significant effect on the quality of skipjack. The TVB content of chilled skipjack with and without dry ice was 33 mg N and 37 mg N, respectively.

Unlike milkfish and skipjack, the use of ice and dry ice for chilling/transportation of shrimp was found to be more effective. The use of the same proportion of shrimp:ice:dry ice could reduce the temperature in the centre of the shrimp muscle to as low as 2.7°C as compared to 6.1°C and 10.4°C for milkfish and skipjack, respectively. Results also indicated that the use of ice was only effective in maintaining the chilled temperature up to 11 h of transportation; the incorporation of dry ice further prolonged the chilling effect of ice. The incorporation of dry ice could maintain the lowest temperature of the centre of the shrimp for up to 15 h, as compared to 7 and 5 h for milkfish and skipjack, respectively. Chemical analysis also indicated that the use of dry ice could improve the shelf-life of chilled shrimp as evident from the lower TVB content (about 18-19 mg N) compared to control samples (21.5 mg N).

Results showed that the pattern of temperature changes in common carp during chilled transport were similar to that of milkfish, skipjack and shrimp. The temperature of common carp rapidly declined during the first 4 h, but gradually increased thereafter. Common carp chilled with ice and dry ice were still in a good condition after 24.5 h of transport, while signs of quality deterioration were evident for samples without dry ice.

About 5 kg of live common carp with an average weight of 250-300 g were used in this study. The amount of sawdust, ice and dry ice used was 10 kg, 2 kg and 2 kg, respectively. Results showed that common carp could be transported alive without water up to 5 h at a survival rate of about 60%. There was also evidence that the incorporation of dry ice could maintain the temperature of sawdust between 9-12°C, which otherwise tends to fluctuate widely during transport.

Further studies are obviously needed to improve survival rates and practicability for commercial application, since these results are still inferior compared to the live shrimp transport experiment using chilled sawdust conducted by Cholik (1982) and Salehudin (pers. comm).

This study on transportation of fresh fish using bamboo baskets was intended to improve the current practice of fish transport using non-returnable containers, since insulated boxes are more expensive and sometimes posed technical and financial problems when returning to owners. About 48 kg of milkfish packed with 12 kg ice and 4 kg dry ice were used in this study respectively.

Results showed that after 30 h of chilled transport, the temperature of the centre of fish muscle in the top layer was around 5°C, whereas that in the middle of the basket was between 10-11°C. At the end of the transport, 300 g of dry ice and 2.5 kg ice were still left over. The pattern of temperature changes in the case of milkfish during transport showed that the incorporation of dry ice could maintain the chilled temperature of about 5°C which is quite ideal for chilled transport. Ice was found effective only up to 8 h with the average temperature at the centre of the fish muscle at 10°C, followed by a gradual increase to 20-21°C after 25 h. The quality of milkfish packed in ice-dry ice mixture at the end of transportation was found to be in good condition

Subject to further commercial-scale studies, therefore, the incorporation of dry ice into chilled transport of milkfish using bamboo baskets seems to be promising. The results of the study also support the findings of Dholakia et al. (1982), which indicated that the use of dry ice for long distance transport (up to 1,200 km) of frozen seafoods appeared to be promising.

Conclusion

The following conclusions are drawn from this study:

- The incorporation of dry ice could significantly reduce the amount of ice to 1:4 (ice:fish), and therefore, the cooling efficiency of ice as well as the amount of fish which could be transported were improved. The extension of the cooling effect of ice through the incorporation of dry ice is more prominent for bulk handling/ transport of more than 500 kg of fish.
- With the incorporation of dry ice, the distance covered could be significantly extended.
- The incorporation of dry ice seems to be promising especially as an anaesthetic agent as well as a means to maintain low temperatures in live fish/shrimp transport.
- Further studies are needed particularly on the economic impacts of dry ice incorporation in commercial application of fresh and live fish handling.

References

Barnett, J. 1989. Fears of flying: How you can guard against the pitfalls of air freighting. Seafood Supplier, August, 1989.

Boeri, R.L., Davidovich, L.A., Giannini, D.H. and Lupin, H.M. 1985. Method to estimate the consumption of ice during fish storage. Int. J. Refrigeration, (8):97-101.

Cholik, F. 1982. Studies on Live Transportation of Shrimp Broodstock Using Chilled Sawdust. Research Institute of Coastal Aquaculture, Maros, Indonesia.

Dholakia, A.D., Khatri, C.N., Trivedi, Y.A., Dabhi, K.G. and Chhaya, N.D. 1982. Preliminary studies on utilisation of dry ice in long distance transportation of frozen seafoods. In: Symposium on the Harvest and Post-harvest Technology of Fish, Cochin, India, November 1982.

Putro, S. 1989. Dry ice - Possible use in fresh and live fish handling. INFOFISH Int., No. 4/89, p 24-25.

Salehudin, M. 1989. Preliminary Study on Live Transportation of Shrimp Using Chilled Sawdust. Personnal communication.

BROWNING OF SALTED SUN-DRIED FISH

G. Smith and M. Hole
School of Food and Fisheries, Humberside College of Higher Education, Nuns Corner, Grimsby, S. Humbs., U.K.

Summary

The lipid of traditional salted, sun-dried fish is highly susceptible to oxidation during processing and storage at tropical ambient temperatures (25-30°C), leading to browning and potential loss of nutritional and economic value of the product. Determinations of extractable fluorescence and soluble brown colour have been found to be relevant indicators of the degree of lipid oxidation in this salted-dried fish. Studies of model systems consisting of aerated fish oil and a range of natural components of fish at 25°C confirmed that the products from lipid oxidation reacted with phospholipids and amino acids to produce fluorescence. Similarly, proteins and amino acids interacted with lipid oxidation products to produce browning, although this only occurred at 25°C in the presence of water. Temperatures above 50°C are required for the development of browning of fish oil alone. The level of the amino acids in salted, sun-dried fish were found to decrease during storage which correlates with their involvement in fluorescence and colour production. The fluorescence/colour can be related mechanistically to the development of lipid oxidation products and hence provides a realistic basis for their acting as indicators of extensive lipid oxidation.

Introduction

Enzymic and non-enzymic processes may be responsible for the browning which arises during the processing and/or storage of many different foodstuffs and may be considered desirable or undesirable depending on the particular product. Although enzymic (polyphenoloxidase) reactions are responsible for colour production in shellfish (Cobb, 1976), it is unlikely that this is the case in processed/stored finfish. The non-enzymic browning associated with salted-dried fish leads to a loss in economic value and hence is regarded as undesirable (Esser et al., 1987). The types of reaction which contribute to non-enzymic browning include the caramelisation of sugars and other carbohydrates, the Maillard reaction between amino acids and sugars, and the interaction between oxidised lipids and proteins.

Early reports on the browning of dried fish suggested that it was a consequence of sugar (ribose) - amino reactions (Tarr, 1954; Jones, 1962). Shewan (1955) noticed a correlation between the degree of browning and copper content of the salt used in salted fish. The browning of fish muscle has also been suggested to arise from a two stage reaction involving oxidised lipids (Pokorny, 1981). He described the first stage as the interaction of the decomposition products of hydroperoxides, particularly carbonyl compounds, with free amino groups of proteins and nitrogenous groups of phospholipids, to form light coloured fluorescent intermediates, chiefly Schiff bases. These are transformed in the second stage to form brown macromolecular products also known as melanoidins.

Work on monitoring lipid oxidation in salted-dried fish (Smith et al., 1989) showed that determination of fluorescence and soluble colour were more suitable indicators of extensive rancidity than the more usual peroxide (PV) and thiobarbituric acid (TBA) values in products which had been stored at tropical ambient temperatures (25-30°C) for up to 3 months. Subsequent work was involved with the characterisation of the reactions contributing to the development of fluorescence and brown colour in salted-dried fish at these temperatures (Smith, 1988). Model systems of fish oil (MaxEPA) were aerated at 25 and 50°C with and without addition of amino acids, protein, phospholipid and water, in a Rancimat apparatus (Rancimat 617, Metrohm). Oil contained in reaction cylinders was flushed with air (2-3 L/h) for up to 10 days. Samples of oil were removed at regular intervals and analysed for peroxide value, acetic acid soluble colour (400 nm) and organic soluble fluorescence (Ex362 nm, Em440 nm). Volatile acids which arise during oxidation were automatically distilled into water and their level recorded conductometrically to give an induction period.

Results and discussion

Effect of temperature

Aeration of fish oil at 25°C gave an induction period of 60 h and a maximum peroxide value of over 3,000 (Figure 1). Development of colour and fluorescence were not observed in this trial. An increase in the temperature of oxidation to 50°C did allow the formation of fluorescence and browning in the oil (Figure 2). This was accompanied by a shortening of the induction period and a lowering of the maximum peroxide value. Thus an increase in temperature from 25 to 50°C leads to an increase in the rate of lipid oxidation, facilitating hydroperoxide breakdown to produce conjugated, possibly aromatic, systems which show fluorescence and browning. These will not be Schiff base type compounds as no nitrogenous groups are present in the oil, i.e. MaxEPA is a refined oil consisting of triglycerides with no phospholipids. Salter (1986) noticed similar effects on the aeration of MaxEPA at 90 and 120°C.

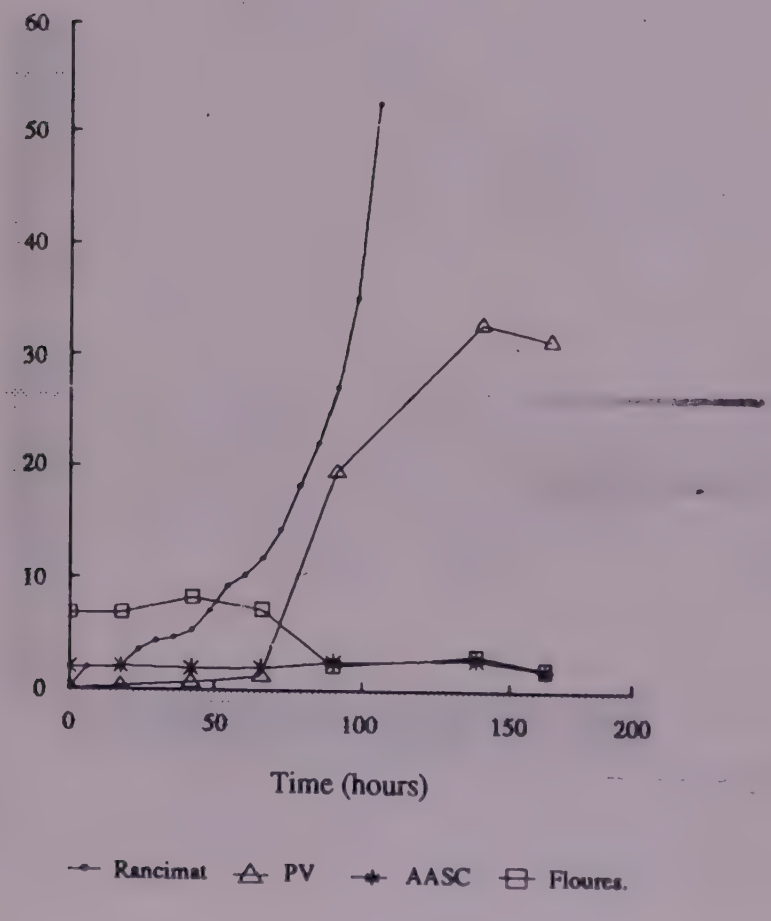

Figure 1. Oxidation of MaxEPA at 25°C

A value of 50 for the Rancimat trace is equivalent to a deflection of 300 uS/cm. Units for peroxide value (PV) are 0.01 x mEq/kg lipid; acetic acid soluble colour (AASC) - absorbance units/g lipid/ml, flouresoence (chloroform/methanol extracts) - relative emission/g lipid/ml.

The runs were performed in triplicate and the average percentage coefficients of variation were 19.7, 12.1, 11.4 and 14.8 for the Rancimat trace, PV, AASC and flouresoence respectively.

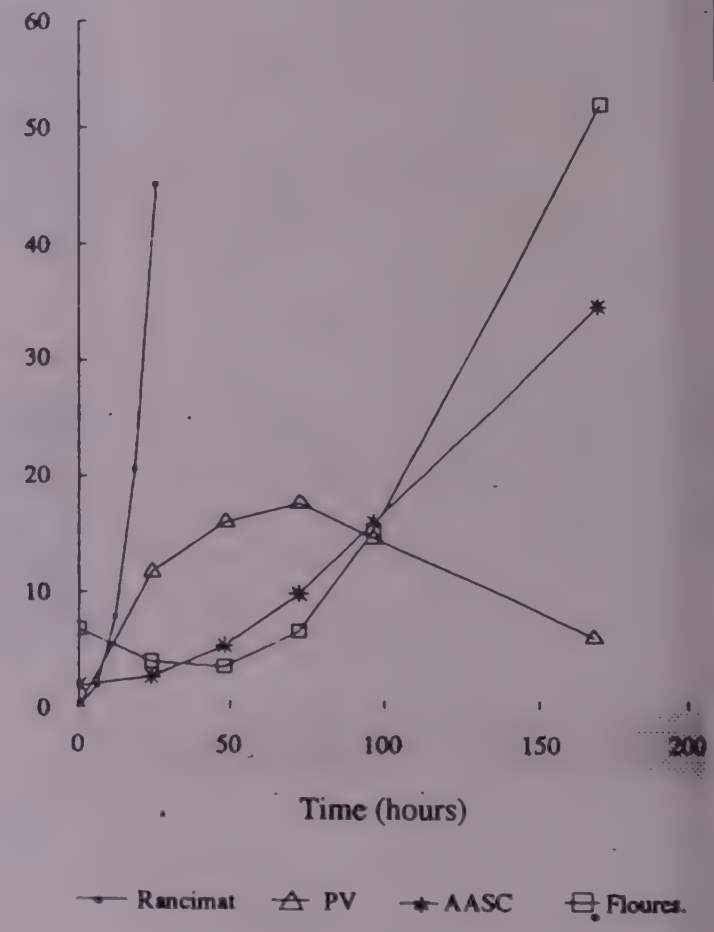

Figure 2. Oxidation of MaxEPA at 50°C

A value of 50 for the Rancimat trace is equivalent to a deflection of 300 uS/cm. Units for peroxide value (PV) are 0.01 x mEq/kg lipid; acetic acid soluble colour (AASC) - absorbance units/g lipid/ml; flouresoence (chloroform/methanol extracts) - relative emission/g lipid/ml.

The runs were performed in triplicate and the average percentage coefficients of variation were 16.0, 11.5, 9.9 and 7.7 for the Rancimat trace, PV, AASC and flouresoence respectively.

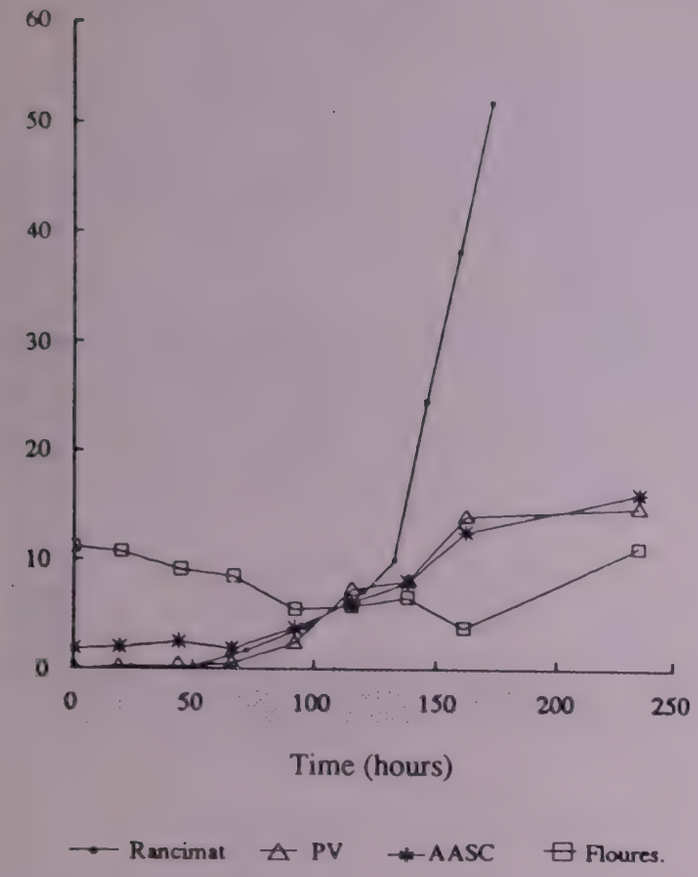

Figure 3. Oxidation of MaxEPA at 25°C with addition of 1% phospholipid

A value of 50 for the Rancimat trace is equivalent to a deflection of 300 uS/cm. Units for peroxide value (PV) are 0.01 x mEq/kg lipid; acetic acid soluble colour (AASC) - absorbance units/g lipid/ml; fluorescence (chloroform/methanol extracts) - relative emission/g lipid/ml.

The runs were performed in triplicates and the average percentage coefficients of variation were 40.5, 26.2, 30.9 and 18.9 for the Rancimat trace, PV, AASC and flourescence respectively.

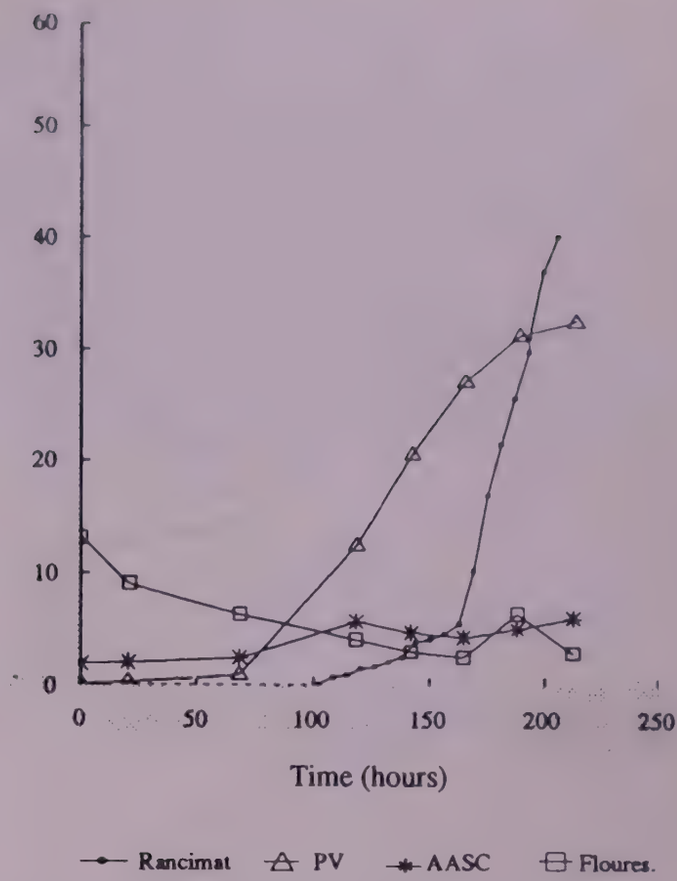

Figure 4. Oxidation of MaxEPA with addition of 1% protein

A value of 50 for the Rancimat trace is equivalent to a deflection of 300 uS/cm. Units for peroxide value (PV) are 0.01 x mEq/kg lipid; acetic acid soluble colour (AASC) - absorbance units/g lipid/ml; fluorescence (chloroform/methanol extracts) - relative emission/g lipid/ml.

Fluorescence and browning were observed in salted-dried fish which had been processed and stored at temperatures around 25°C. It does not appear from the above results that these phenomena are produced from oxidation of the fish oil alone.

Addition of phospholipids

Addition of egg yolk lecithin to fish oil aerated at 25°C resulted in a lengthening of the induction period, a lower maximum peroxide value and production of a small amount of fluorescence and colour (Figure 3). The nitrogenous groups present in this type of lipid are able to react with unsaturated carbonyl compounds (from lipid oxidation) to produce the fluorescent Schiff base type compounds and ultimately colour in the oil, as described by Dillard and Tappel (1973).

Incorporation of water to the fish oil and phospholipid system, at levels found in salted-dried fish, had no additional effect on the oxidation at 25°C.

Addition of protein

Fish oil containing 1% by weight of lysozyme was aerated and after 9 days, no significant production of fluorescence and colour was observed (Figure 4). Reports of browning upon reaction of proteins with oxidised lipids (Pokorny et al., 1974; El-Zeany, 1975; El-Zeany and El-Tarras, 1976) have all involved experiments conducted at 60°C, but the results here show that a temperature of 50°C leads to browning of fish oil alone.

A fish oil system containing protein and in addition, water formed an emulsion which did become brown after 6 days aeration. Upon separation of the emulsion layers, it was observed that all the brown colour was contained in the aqueous layer, with only very slight browning of the oil. This confirms the findings of El-Zeany (1975) who reported that water soluble products arose from the reaction of unsaturated aldehydes with protein.

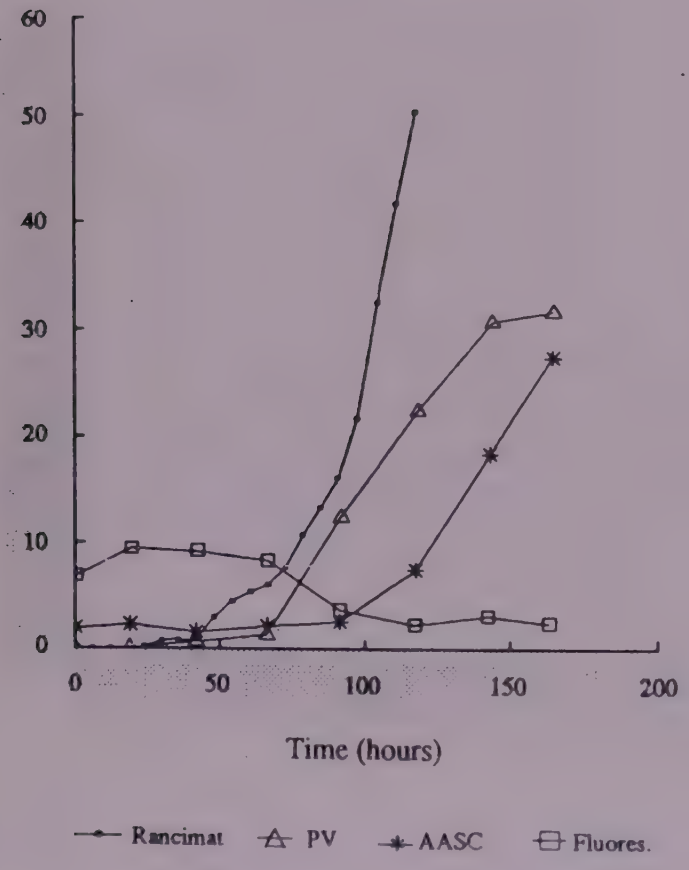

Figure 5. Oxidation of MaxEPA with addition of 1% glycine

A value of 50 for the Rancimat trace is equivalent to a deflection of 300 uS/cm. Units for peroxide value (PV) are 0.01 x mEq/kg lipid; acetic acid soluble colour (AASC) - absorbance units/g lipid/ml; fluorescence (chloroform/methanol extracts) - relative emission/g lipid/ml.

The runs were performed in triplicate and the average percentage coefficients of variation were 42.6, 14.1, 18.6 and 13.2 for the Rancimat trace, PV, AASC and fluorescence respectively.

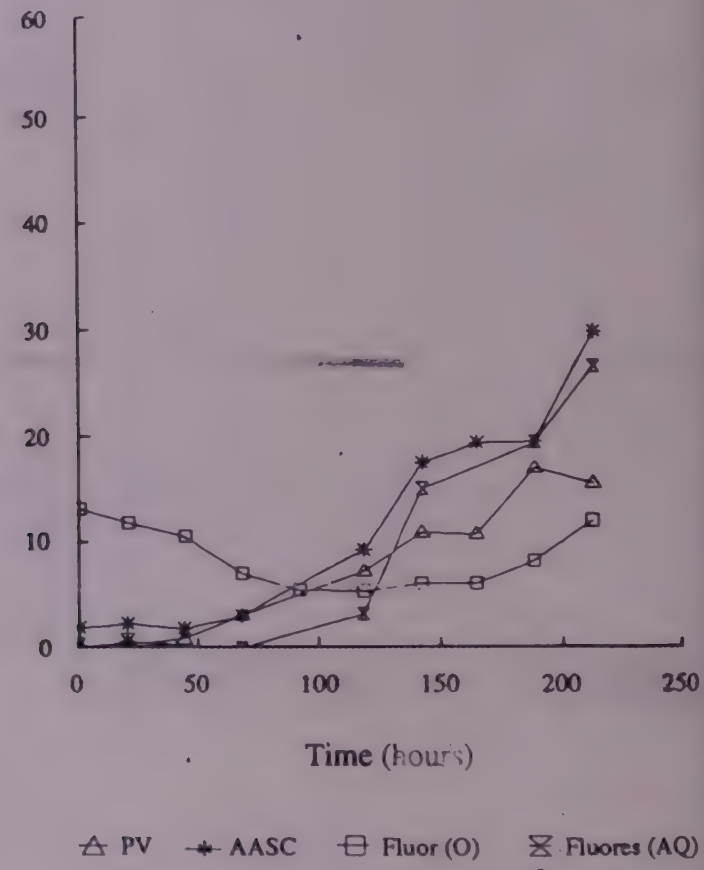

Figure 6. Oxidation of MaxEPA with addition of 1% glycine + 50% water

Units for peroxide value (PV) are 0.01 x mEq/kg lipid; acetic acid soluble colour (AASC) - absorbance units/g lipid/ml; fluorescence (chloroform/methanol and aqueous extracts) - relative emission/g lipid/ml.

The runs were performed in triplicate and the average percentage coefficients of variation were 52.3, 52.8, 20.3 and 53.2 for PV, AASC, chloroform/methanol soluble fluorescence and aqueous soluble fluorescence respectively.

Addition of amino acids

The results in Figure 5, for aeration of fish oil and glycine, show a close similarity to those obtained from the oxidation of fish oil alone (Figure 1) apart from values of acetic acid soluble colour, although this browning only occurred upon addition of acetic acid (a polar solvent) to the oil/glycine mixture, and was not a property of the oil itself, i.e. the oil did not change colour during the aeration. Similar results were also obtained with lysine in place of glycine. El-Zeany (1975) demonstrated browning in mixtures of unsaturated aldehydes with glycine and lysine, reacted at 60°C. Oxidation of the fish oil alone at 50°C (Figure 2) resulted in browning of the oil, thus addition of amino acids should likewise result in browning at this temperature.

Aeration of a MaxEPA system containing glycine and water (Figure 6) demonstrated that decomposition of hydroperoxides to give fluorescent and coloured compounds has occurred, with browning and fluorescence of both lipid and aqueous phases. Thus this investigation shows that the addition of water, as a polar solvent is required in this system for the reaction between amino and carbonyl groups to form a Schiff base, as shown in Figure 7. This is in contrast to the MaxEPA/phospholipid system in which fluorescence developed without the presence of water.

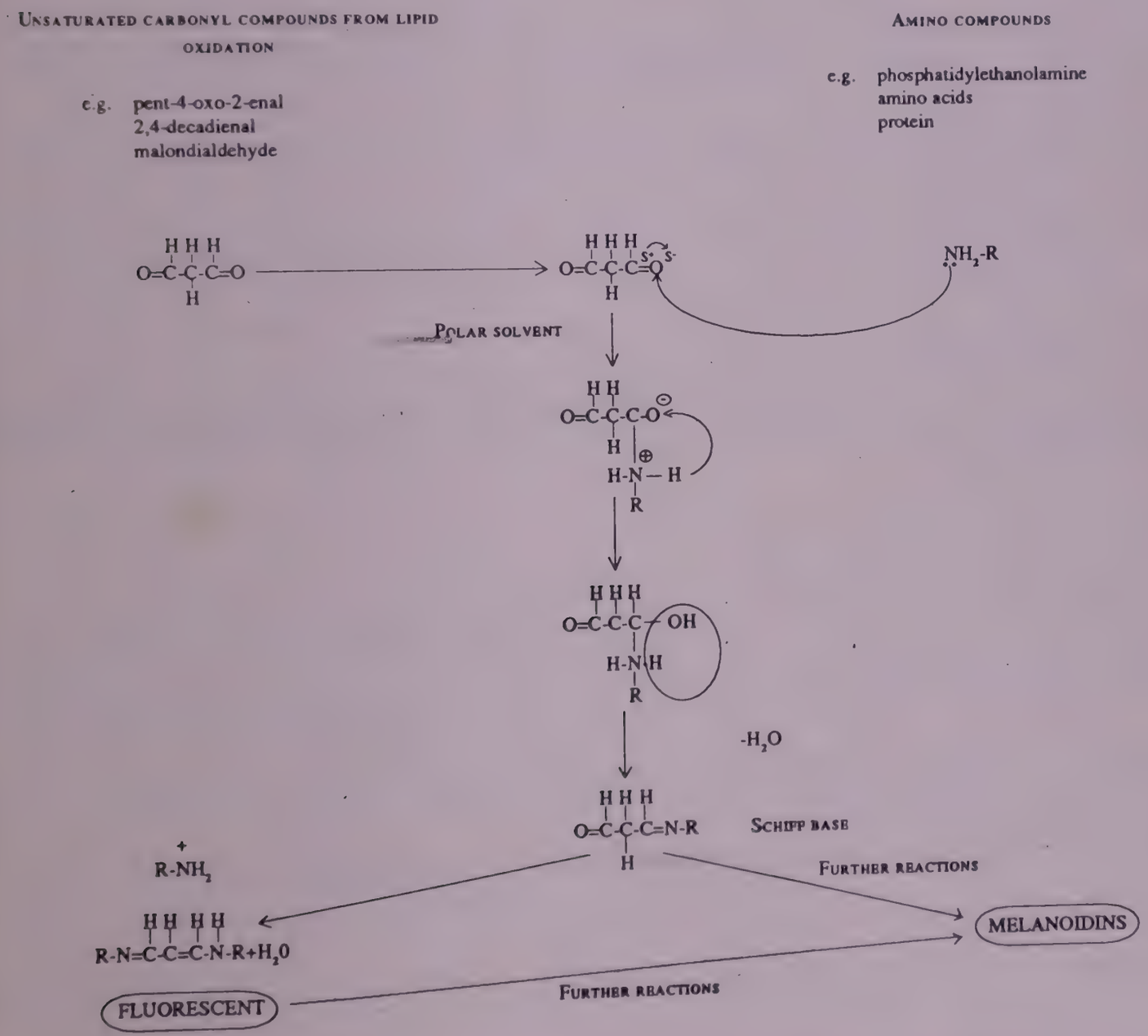

Figure 7. Reactions producing fluorescence and colour during lipid oxidation

Studies on the free amino content of salted-dried catfish have shown that up to 11.4% of the protein consisted of free amino acids, the levels decreasing to 5.9% of the protein after 3 months storage at tropical temperatures (Smith et al., 1989). This loss was accompanied by a rise in values of soluble colour and fluorescence which points to a relationship between free amino acids and the development of browning in salted-dried fish.

Conclusion

In the polar environment of salted-dried fish, reactions between lipid oxidation products and the amino groups of amino acids or phospholipids will result in fluorescence which may be used as a more relevant indicator of extensive lipid oxidation than peroxide or TBA values. Despite high levels of peroxides and carbonyls being recorded during the processing of the fish, as also observed in the model systems, the values become negligible in the stored fish product. Additionally, reactions between lipid oxidation products and amino acids or proteins contribute to the browning of this type of fish product.

References

Cobb, B.F. 1976. Biochemistry and physiology of shrimp - effect on use as food. In: Proceedings of the Conference on the Handling, Processing and Marketing of Tropical Fish. 405- 411.

Dillard, C.J. and Tappel, A.L. 1973. Fluorescent products from reaction of peroxidising polyunsaturated fatty acids with phosphatidyl ethanolamine and phenylalanine. Lipids, 8:183-189.

El-Zeany, B.A. 1975. Oxidised lipids-proteins browning reactions. I. Reaction of aldehydes with amino acids and proteins. Egyptian J. Fd. Sci., 3:81-89.

El-Zeany, B.A. and El-Tarras, M.F. 1976. Oxidised lipids-browning reactions. II. Effect of the protein substrate. La Rivista Italiana Della Sostanze Grasse, 53:175-177.

Esser, J.R., Hanson, S.W., Rosenthal, A.J., Tausin, S. and Harijanto 1987. Report on the Training Programme carried out in East Java during October and November 1986. Overseas Development Administration, London and Directorate General of Fisheries, Jakarta.

Jones, N.R. 1962. Browning reactions in dried fish products. In: Recent Advances in Food Science, J. Hawthorne and M. Heikch (eds.), Butterworth, London, pp. 74-79.

Pokorny, J. 1981. Browning from lipid-protein interactions. Progress in Food and Nutrition Science, 5:421-428.

Pokorny, J., El-Zeany, B.A., Kolakowska, A. and Janicek, G. 1974. Non-enzymic browning. IX. Correlation of autoxidation and browning reactions in lipid protein mixture. Zeitschrift fur Lebensmittel-Untersuchung und Forschung, 157:323-326.

Salter, R.M. 1988. Analysis of Food Lipid Material using Capillary Gas Liquid Chromatography. Ph.D. Thesis. CNAA, Humberside College of Higher Education.

Shewan, J.M. 1955. The browning of salt cured white fish. Food Manufacture, May, 200-203.

Smith, G. 1988. Lipid Oxidation in S.E. Asian Salted-Dried Fish. Ph.D. Thesis. CNAA, Humberside College of Higher Education.

Smith, G., Hole, M. and Hanson, S.W. 1989. Assessment of lipid oxidation in Indonesian salted-dried marine catfish. J. Sci. Fd. Agric., In Press.

Tarr, H.L.A. 1954. The Maillard reaction in flesh foods. Fd. Technol., January, 15-19.

ASSESSMENT AND REDUCTION OF INSECT INFESTATION AND LOSSES OF CURED FISH IN INDONESIA, A CASE STUDY

J.R. Esser, Sunarya** and J. Wiryante***

*School of Food Studies, Humberside College of Higher Education, Nuns Corner, Grimsby DN34 5BQ, United Kingdom

**Directorate General of Fisheries, Jl. Harsono Rm. No. 3, Ragunan, Ps. Minggu, Jakarta Selatan, Indonesia

Summary

Curing by salting and drying is a major method of fish preservation in Indonesia. Official sources estimate the total amount of fish destined for drying to be in excess of 700,000 tonnes per year and it is predicted that cured fish will continue to be a nutritionally and economically important commodity in the foreseeable future.

Cured fish is highly susceptible to insect infestation during processing and storage, which results in considerable physical and financial losses. Many fish processors have responded to the problem by illegally applying household insecticides to fish during processing. Since 1982, Humberside College of Higher Education, the Indonesian Directorate General of Fisheries and the Overseas Development Natural Resources Institute have collaborated in Overseas Development Administration/Government of Indonesia funded projects, aimed at assessing and developing methods of reducing these losses. An important finding of this work, was that the FAO/WHO approved insecticide, pirimiphos-methyl is an effective and financially viable solution to the insect infestation problem and represents the only safe remedy that is likely to be successfully adopted by fish processors in the near future.

This paper outlines the development of the project work in Indonesia and details a recently conducted blowfly trial to illustrate the field methods used in evaluating the effectiveness of insecticides as protectants of cured fish against insect infestation during processing. Possible areas of future research into the reduction of post-harvet losses of cured fish are also discussed.

Introduction

It is predicted that increasing demand for fish, at a time when many fish stocks are being fully or overexploited, will result in a short-fall in supply of at least 10 million tonnes by the year 2000 (Whittle, 1985). This short-fall can only be met by improved utilisation of the catch, an important aspect of which is the reduction of post-harvest losses, which are thought to account for 10% of total world fish production and are believed to be highest in small scale fisheries, where fish are processed by traditional methods such as salting, drying and smoking.

Few detailed studies have been conducted into post-harvest losses of cured fish, particularly in Southeast Asia, but losses of 25% are thought to be common and in some instances estimates as high as 50% have been made (Poulter et al., 1988).

Fish curing is achieved by salting, drying and smoking, or a combination of these treatments. Although processing techniques show considerable variation, they all extend the shelf-life of the product through reducing water activity (Aw) and consequently inhibiting microbial spoilage.

Traditional fish processing is often conducted under basic, unhygienic conditions and is subject to a number of constraints that limit the quantity and quality of fish produced by the processors. Insect infestation is often regarded as the major problem experienced by cured fish processors, many of whom have resorted to applying household insecticides to their fish, in order to reduce infestation and damage. Such widespread abuse of insecticides by cured fish processors illustrates the urgent need for the introduction of safe, alternative method of infestation reduction.

Project overview

The project commenced in 1982, when an initial survey of traditional fish processing was conducted in Thailand, Burma, Malaysia, Indonesia and the Philippines. Futher surveys were conducted in Indonesia and Thailand between 1983 and 1989. The principal aims of the surveys were to identify and assess the problems being experienced by cured fish processors, wholesalers and retailers. At each location, special attention was given to details of processing technique, fish species being processed, situation and standard of hygiene of the premises, nature of insect attack, methods used to deter insect attack and magnitude of financial and physical losses. Information was obtained by using a combination of direct observation and interviews using unstructured questionnaires. Insect samples were collected for identificaton and cured fish samples were taken for proximate analysis.

The surveys revealed widespread cured fish losses, both physical and financial, in the countries visited and identified a lack of quantitative data on these losses. Processors, wholesalers and retailers reported physical and financial losses of between 10-50% and 25-90% respectively. These variations arose from differences between processing methods, standards of hygiene, species processed, storage and distribution regimes and seasonal effects. Blowfly infestation was identified as the major cause of losses during processing. Examination of over 3,000 blowfly specimens, collected from fish at 20 processing sites in 10 locations in Indonesia and Thailand, showed that one species, *Chrysomya megacephala* (Fabricius) was overwhelmingly involved. Losses during storage were principally due to infestation with *Dermestes spp.* and *Piophila casei* (L.). Fragmentation, rancidity, microbial spoilage and rodent attack were also identified as important causes of losses. The illegal use of household and agricultural insecticides such as Baygon, Startox, Mafu, Swallow, Sevin, Lebaycid, Dipterex and Neguvon as protectants of cured fish was widespread and illustrated the urgent need for the development of safe, practical alternative methods of reducing infestation.

Subsequent to the initial survey, field investigations into quantitatively assessing losses and loss reduction techniques were conducted at the premises of a small scale fish processor located in Cirebon, West Java. The early trials evaluated the effectiveness of screening, extended salting and treatment of fish with pyrethroid insecticide Fastac (active ingredient alpha cypermethrin) in protecting fish against insect infestation during processing and storage. The results of these trials, which are described in Esser et al. (1985), demonstrated that the simple technique of fitting a lid to the salting tank prevented infestation during salting. The screen used to protect the fish during drying effectively prevented infestation but was found to be impractical in use. Extended salting of the fish was ineffective in controlling blowfly infestation and laboratory investigations into the effects of salt concentration on the principal blowfly pest, *Chrysomya megacephala*, confirmed that far higher salt concentrations than are usually found in traditionally processed fish are necessary to control infestation (Esser, 1988). Treatment with the insecticide Fastac was found to be very effective at controlling blowfly and dermestid beetle infestation when applied at very low concentrations. At the time of the trials, the only insecticide which had FAO/WHO approval for use on fish was pyrethrum synergised with piperonyl butoxide. However, this insecticide only gave adequate protection when applied at concentrations which left unacceptably high residues in the fish (FAO, 1981). Although Fastac was demonstrated to be a highly effective protectant when applied at very low concentrations, it did not have FAO/WHO approval for use on fish. The insecticide approval process is, of necessity, very protracted and registration of an insecticide for use in a given commodity can take many years. In view of the urgency for the introduction of a safe, effective alternative to the widespread, illegal practice of treating fish with household insecticides, it was decided in 1984 to commence field trials in Indonesia to evaluate the organophosphate insecticide pirimiphos-methyl, which at that time was being considered for approval for use on fish by FAO/WHO. Approval of pirimiphos-methyl for use on fish was obtained from FAO/WHO in 1986. The pirimiphos-methyl evaluation trials demonstrated that a 0.03% a.i. dip treatment, for a period of fifteen seconds, was sufficient to control insect infestation during processing and storage, while leaving insecticide residues which were within the FAO/WHO M.R.L. of 10 mg/kg (Esser et al., 1986). Numerous trials conducted in Indonesia (Esser et al., 1988) and Thailand (Rattagool et al., 1988) have consistently demonstrated pirimiphos-methyl to be effective in controlling blowfly infestation during processing and dermestid beetle infestation during storage.

In 1986, an Overseas Development Administration/Government of Indonesia funded project, aimed at providing provincial fisheries quality control staff with training in post-harvest loss assessment and reduction techniques commenced in Indonesia. Training programmes were conducted in nine provinces of Indonesia and each programme consisted of seminars, field trials and survey work. The loss reduction training concentrated on the application and evaluation of pirimiphos-methyl as a protectant against insect infestation, in anticipation of pirimiphos-methyl receiving approval for use on fish by the Indonesian authorities. However, it was not possible to recommend, or provide training in the use of pirimiphos-methyl to the processors.

until the necessary clearances had been obtained. Pirimiphos-methyl received provisional registration for use on fish in Indonesia in February 1988 and is marketed by PT ICI Indonesia as Minawet 250 EC.

During the final phase of the training project, a financial evaluation of the use of Minawet was conducted at four processing sites in Indonesia (Gordon and Esser, 1989). This evaluation was conducted to determine the comparative costs of using pirimiphos-methyl and the household insecticide Startox, before making recommendations on a strategy for extending the use of pirimiphos-methyl to fish processors. Startox is commonly used on fish by processors even though it is not formulated for human consumption and is potentially extremely dangerous when misused on food. Although the public health argument alone should be sufficient to bring about a change to pirimiphos-methyl, this ignores the financial implications to the processors, who processes fish to make money. The prospects of a safe alternative technique being successfully adopted by processors will be greatly enhanced if the technique does not confer a perceived financial disadvantage to the processor. The trials demonstrated that pirimiphos-methyl was technically at least as effective as the Startox and, when used correctly, was simpler and marginally cheaper to apply. Moreover, it was found that the cost of applying pirimiphos-methyl was insignificant in relation to other processing costs. Pirimiphos-methyl was therefore demonstrated to be a financially viable, as well as a safe and technically effective alternative to the potentially dangerous household insecticides currently being used by many processors.

Technical evaluation of insecticides as protectants against blowfly infestation of fish during processing

Introduction

The experimental design used in field testing the efficacy of any potential infestation/loss reduction technique must take into account and respond to the nature of the fish processing operation, species processed, processing method and the constraints under which the processor operates, for the results to be of relevance to the commercial fish processor. Without a good understanding of the processing operation, criteria used to determine the success of a particular loss reduction technique might be incomplete or inappropriate. This important information can only be obtained by working with and gaining the trust of processors over a period of time. In addition, resources available to the investigator, such as funding, manpower, time, analytical equipment and the location of the fish processing premises must be taken into consideration. It should be realised, that there is no single experimental design universally appropriate to loss assessment and reduction of cured fish. The experimental design adopted by an investigator must take into account the situation in the field.

To illustrate the experimental design which evolved during the course of investigations into reducing losses of *Arius thalassinus* (marine catfish), a trial conducted at the premises of a small scale fish processor in Jakarta, Indonesia during June 1989 is described below. During this trial, the efficacy of the FAO/WHO approved insecticide pirimiphos-methyl was compared with the household insecticide Startox, as part of a financial evaluation aimed at comparing the costs of the two treatments, prior to making recommendations on an extension strategy for pirimiphos-methyl. It is important to emphasise, however, that the use of Startox on fish destined for human consumption is an illegal and potentially highly dangerous practice, which can under no circumstances can be condoned. At the end of the evaluation trial, the Startox treated fish were destroyed to ensure they did not enter the marketing chain.

Fish processing method and experimental design

The fish used for this trial was *Arius sp.* (marine catfish), which is a species that is highly susceptible to blowfly infestation during processing. On arrival at the processor's premises, the fish were labelled with a unique code number, weighed, beheaded and gutted. The fish were then submerged in water and allowed to ferment overnight. After fermentation, the fish were layered with salt and submerged in saturated brine. To prevent infestation during salting, the entrance to the salting tank was protected by a closely fitting lid. After salting, the fish were split and the abdominal membranes removed. They were then washed and stratified according to weight into three treatments, each treatment consisting of three replicates of ten fish of similar weight ranges. The pirimiphos-methyl (brand name Minawet) treatment fish were individually submerged in 10 L of 0.03% a.i. pirimiphos-methyl emulsion for 15 sec. The control fish received no further treatment. The Startox treatment fish were painted with a Startox-water mixture at intervals during drying, as was the processor's usual practice. The replicate trays of fish were

arranged into three blocks on the drying racks. Each block consisted of one replicate of each treatment and the trays were arranged in a pattern which ensured that "clumping" of trays of the same treatment did not occur. On the second drying day, the fish were split once more and the newly exposed flesh was painted with pirimiphos-methyl or Startox as appropriate. A record was kept of the qualities of insecticide applied to both the pirimiphos-methyl and Startox treated fish. The fish were occasionally turned during drying, and were judged by the processor to be sufficiently dry after 5 days. They were then weighed and inspected for blowfly damage. The damaged areas of heavily infested fish were cut out by the processor and disposed of. The remaining pieces of undamaged fish were weighed once more to obtain the market yield.

Infestation and loss assessment methods

Blowfly activity was monitored by taking instantaneous counts of adult blowflies which had settled on the drying fish. Visual counts were taken at 15 min. intervals on the first day of drying, when the fish were most attractive to blowflies. Total numbers of flies present on each treatment were recorded. Shade air temperature and humidity were simultaneously monitored, using a Casella whirling hygrometer.

Blowfly oviposition was assessed by counting the total number of egg batches oviposited on each replicate batch of fish during the first drying day.

On the morning of the third day, when infestation was most apparent, each fish was inspected for the presence of blowfly larvae. The level of infestation was graded as follows:

Zero - No blowfly larvae present
Light - Occasional larvae present
Moderate - Numerous larvae, but no feeding packs present
Heavy - Numerous larvae, feeding packs present

Five third instar larvae were removed from each infested control fish and subsequently reared to the adult stage to facilitate identification of the blowfly species responsible for infestation.

At the end of drying, the fish were weighed and graded for blowfly damage as follows:

Zero - No damaged areas
Light - Occasional small lesions
Moderate - Numerous small or single large lesion
Heavy - More than one large lesion present

As most physical losses of edible material result from blowfly larvae consuming the fish during the drying phase of the process, estimates of physical losses during processing were obtained from the total weight yield of each replicate of each treatment at the end of drying expressed as a percentage of weight after salting, i.e.:

$$\frac{\text{Total weight of fish in replicate at end of drying}}{\text{Total weight of fish in replicate after salting}} \times 100$$

The mean processed yield of each treatment was then calculated.

In addition, the market yield was determined by reweighing the fish after tissue damaged by blowfly larvae had been cut out by the processor and was calculated as follows:

$$\frac{\text{Weight of fish in each treatment sent to market}}{\text{Weight of fish in each treatment before processing}} \times 100$$

Results and discussion

The fish weights recorded during processing are given in Table 1. Differences in processed yield became apparent after drying. The control fish gave a processed yield of 43%, while the yield of both the pirimiphos-methyl (Minawet) and Startox treated fish was 55%. The 12% yield difference between the control and insecticide treated fish was caused by blowfly larvae consuming the fish during drying. In addition, the larvae caused considerable damage to the unconsumed tissues, which had to be cut out by the processor before he could sell the fish. This resulted in the control fish having a market yield of only 4% and the insecticide treated fish yielding 29-31%. Clearly, in the absence of insecticide, the processing operation was unviable.

Table 1. Fish weights (kg) recorded during processing

Treatment	Whole weight		After gutting		After salting		After drying	
	Mean	Sum	Mean	Sum	Mean	Sum	Mean	Sum
Control	5.3 (1.7)	160.65	3.4 (1.1)	103.50	3.0 (1.0)	91.25	1.3 (0.5)	39.00
Minawet	5.3 (1.7)	159.75	3.5 (1.1)	104.00	3.0 (1.0)	90.35	1.6 (0.6)	49.55
Startox	5.3 (1.7)	159.70	3.5 (1.1)	104.10	3.1 (1.0)	92.85	1.7 (0.6)	50.95

Each treatment consisted of 30 fish. Standard deviation given in parentheses

Blowfly activity data, recorded during the first drying day, are given in Figure 1. Environmental conditions, which were recorded simultaneously, are given in Figure 2. Blowfly activity over the drying fish showed a marked increase during the afternoon, when overcast conditions developed. The blowfly population was dominated by *Chrysomya megacephala* (Fabricius) and occasional *Lucilia cuprina* (Wiedemann) were present. Mean shade temperature and relative humidity were 30.5°C (range 29-31°C) and 69% (range 64-76%). The blowfly activity and environmental data suggest that exposure to direct sunlight, rather than changes in air temperature or relative humidity, is the more important factor influencing blowfly activity. The high level of blowfly activity was undoubtedly a consequence of the unsanitary conditions prevailing at the present site.

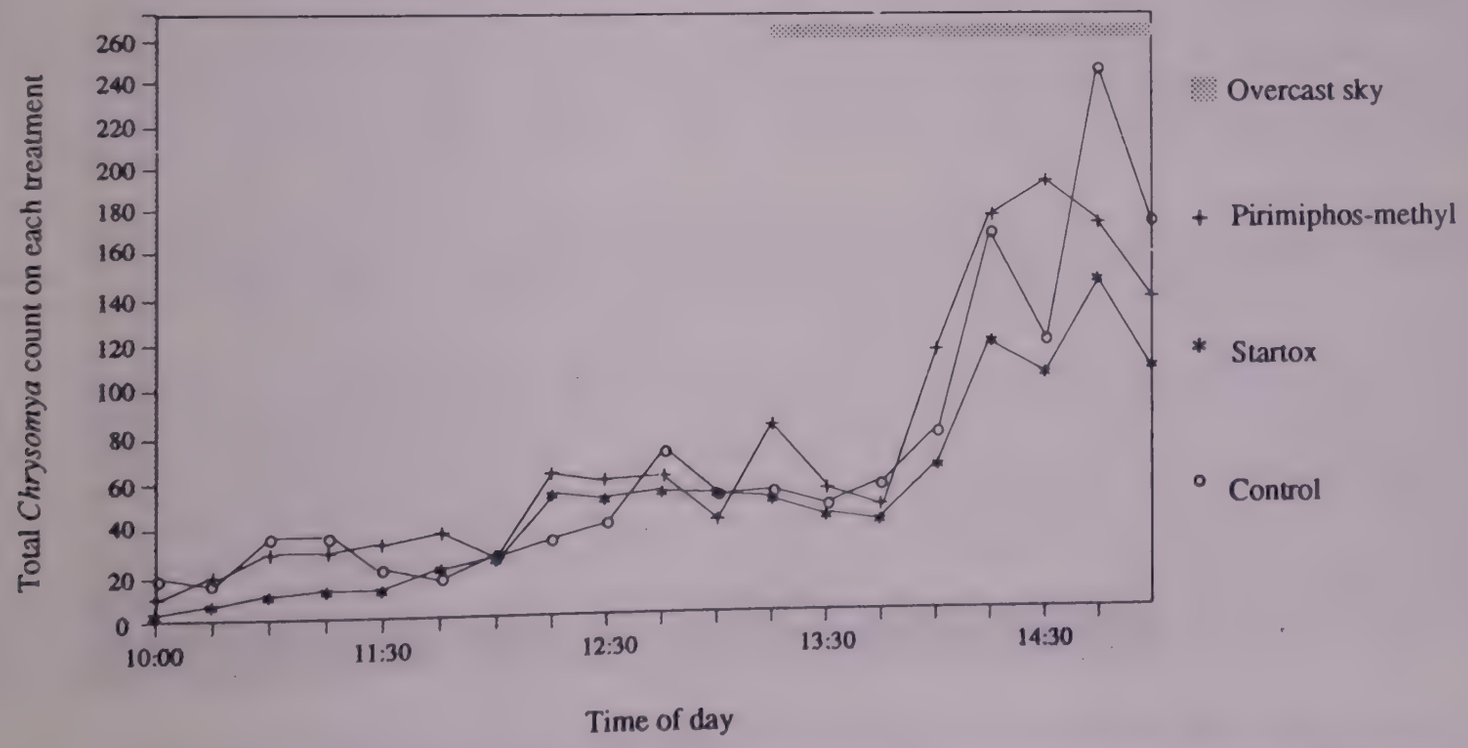

Figure 1. Blowfly counts on drying fish during first drying day (Muara Angke processing site, Jakarta, May 1989)

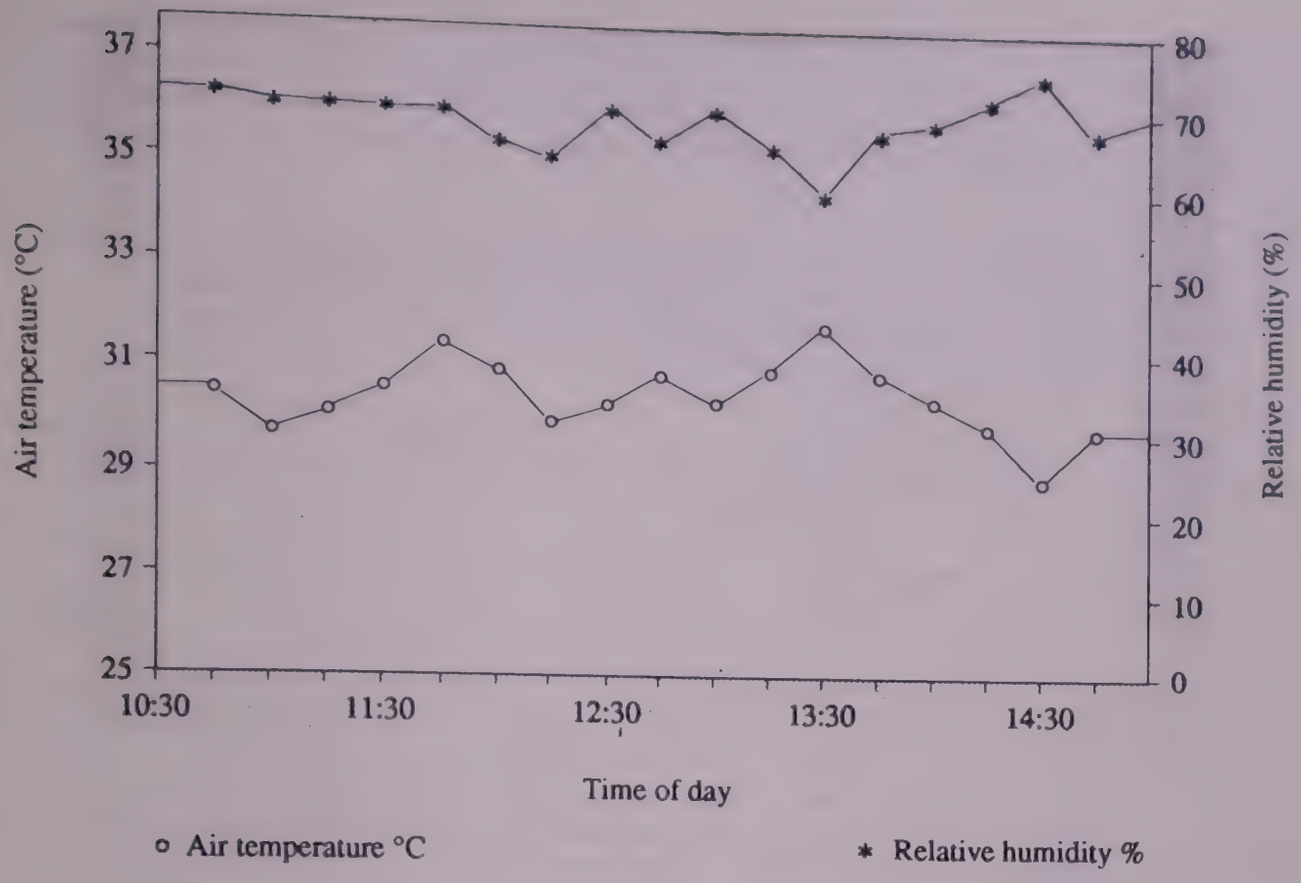

Figure 2. Air temperature and relative humidity during first drying day (Muara Angke processing site, Jakarta, May 1989)

Blowfly oviposition, larval infestation and loss assessment results are given in Table 2. The high level of oviposition during drying was a consequence of the high blowfly activity. The flies preferentially oviposited on the larger fish, where the eggs and larvae would be less liable to dehydration. More egg batches were deposited on the control fish than either of the insecticide treatments. Earlier trials, however, have demonstrated that pirimiphos-methyl does not have a repellent effect on ovipositing blowflies. Both *C. megacephala* and *L. cuprina* were observed ovipositing on the drying fish, although ovipositing *C. megacephala* were present in far greater numbers.

The control fish became heavily infested with blowfly larvae during drying. All of the 118 adult flies reared from larvae collected from the control fish, were identified as *C. megacephala*. Both the pirimiphos-methyl and Startox treatments significantly reduced larval infestation and damage during drying, resulting in increased weight yields.

The infestation and damage data confirmed the results of earlier trials which have consistently demonstrated the effectiveness of pirimiphos-methyl in preventing blowfly infestation during processing. The standard 15 sec., 0.03% a.i. dip leaves residues of less than the FAO/WHO M.R.L. of 10 mg/kg, resulting in a product that is considered safe for human consumption. Although the Startox treatment gave similar protection against infestation, its application presents considerable hazards to both fish processors and consumers. There is risk of acute poisoning by the active ingredients (bioallethrin and dichlorvos) when unmeasured and variable quantities of insecticides are liberally painted on the fish, also there is a potential long term threat to health caused by carcinogens present in the carrying agent. Startox was also found to taint the fish and was more expensive and time consuming to apply. Pirimiphos-methyl, therefore, represents a remedy that can be justified on grounds of safety, cost and ease of use.

The introduction of an insecticide as a loss reduction method, requires very careful planning and organisation, if it is to be successfully adopted and used safely by processors. It is essential that the insecticide is introduced as part of an extension programme aimed at training processors in its correct and safe use. Use of the insecticide by processors needs to be monitored

over a period of time, to ensure that any unforeseen problems are identified and quickly remedied. This would involve regular visits to the processing sites by extension workers trained in loss reduction techniques and insecticide use. In addition, fish samples should be collected at frequent intervals for residue analysis by the appropriate agency, to ensure that residue limits are not being exceeded. Insecticides should not be introduced to processors in the absence of such an extension and monitoring system.

Table 2. Effects of insecticide treatment on blowfly oviposition (total no. egg batches oviposited on each treatment), percentage of fish in each treatment suffering moderate to high (M-H) larval infestation and percentage suffering moderate to high (M-H) larval damage

Treatment	Egg count	% M - H Infestation	% M - H Damage
Control	151	93	100
Minawet	112	7 **	3 **
Startox	10	30 *	3 **

nsd - no statistically significant difference between control and insecticide treatment.
* - p value obtained from difference between control and insecticide treatment < 0.05.
** - p value obtained from difference between control and insecticide treatment < 0.001.

Possible areas of future research

Insect infestation of traditionally processed fish in the tropics will continue to be a serious problem until safe, effective and economic countermeasures are successfully introduced.

Although pirimiphos-methyl appears to fulfil all of the requirements of a successful remedy, the fact remains that it is still a poison and therefore does not represent an ideal answer to the problem. While its current use can be justified on the grounds that it is far safer than some of the insecticides currently being applied to cured fish, it would be unreasonable to regard it as a long-term remedy, while failing to continue research into alternative countermeasures, that do not require the application of insecticides.

Screening is a possible remedy that warrants further investigation. Guarding the salting tank with a closely fitting lid, prevented infestation during salting and was a technique readily adopted by the processor in Cirebon. While the design of the screen, used during the drying trials, presented practical problems and was not adopted by the processors, alternative designs might overcome the problems experienced and should be evaluated. Permanent, walk in designs may be suitable for premises with large drying areas and although initial capital outlay would be high, they should be inexpensive to maintain and, in the long term, would probably pay for themselves. For continued protection during storage, suitable, cost effective storage packaging techniques require further investigation.

The role of volatile chemicals, released by fish during processing, in attracting gravid blowflies is another area that requires further research. Field observations suggest that, at certain stages during processing, fish release a powerful, olfactory stimulus, which results in blowflies being attracted to and subsequently ovipositing on the fish. The isolation and identification of these volatiles could lead to analogues being developed and incorporated into fly traps. In addition, pheromones that elicit group oviposition by blowflies, could be further investigated with the same end in mind. Pheromone baited traps have proved successful in controlling other insect pests (Hodges, 1984) and could be integrated with other methods to control infestation.

Further information on naturally occurring insect repellents/insecticides is also required. Asastyasih and Madden (1986), investigated the role of plant products and extracts in preventing blowfly infestation of salted-dried fish and observed that white pepper, garlic, star fruit extract and acetic acid had a repellent effect on *Musca domestica vicina* (Macquart) and *C. megacephala*.

the Gambia, the use of lime juice and ground chillies, to control blowfly infestation of sundrying fish, has been observed by alker and Evans (1984). Pepper is used by some fish processors in Burma, Malaysia and Indonesia, to control blowfly infestation d its use has also been reported in India by Pillai (1957) and Nigeria by Rollings and Hayward (1963). Don-Pedro (1985) found at powdered sun-dried citrus peel could reduce infestation of dried *Clarius sp.* by *Dermestes maculatus* (Degeer). It must be me in mind, however, that if any of the natural repellents/insecticides, sometimes used by processors, are effective and onomic, then one would expect their use to be more widespread than is apparent. However, it would be worthwhile to identify e active ingredients of these plant products, with a view to developing more effective repellents.

Further investigation of the biological basis of the high salt tolerance shown by *C. megacephala* larvae should provide eful information on insect salt regulation mechanisms. In addition, the nature of the apparent sensitivity to salt of African owfly species, needs to be ascertained.

Large blowfly populations result from poor standards of hygiene and sanitation, which themselves are products of poverty d ignorance. Trial, anti-blowfly campaigns should be carried out to see if it is practical to reduce *C. megacephala* populations, rticularly in the densely populated, urban areas, where fish processing premises are often located.

- To be effective, the campaign would require active collaboration between the various departments responsible for public alth, sanitation and education, if it is to bring about a sustained improvement in standards of hygiene.

Apart from benefiting fish processors themselves, such a campaign would be worthwhile from a general public health int of view, in contributing to an increase in the standard of health of the community and consequently the standard of living d quality of life of the less affluent members of society.

Blowflies are notorious carriers of disease, particularly the pathogens that cause common diseases in developing countries g. diarrhoea, dysentery and cholera. Food poisoning microorganisms such as *Staphylococcus aureus* and faecal indicators, longing to *Enterobacteriaceae* and *Vibrionaceae*, have been isolated from *C. megacephala*, collected at fish processing sites Jakarta (Anggawati et al., 1986). Blowflies are also thought to have a role in the transmission of tape worm eggs, which they ck up when feeding on human and animal faeces (Lawson and Gemmell, 1985).

Identification of the chemicals that attract blowflies could lead to synthetic analogues being used in a blowfly trapping ogramme, as part of the wider campaign to reduce the blowfly population. Such a campaign would be expensive and complicated administer, but the potential benefits, particularly in the general area of improved public health, should more than justify the fort and expense.

Post-harvest losses in cured fish are unacceptably high in tropical countries and can have serious economic and public alth consequences. Unfortunately, for various reasons, cured fish is a relatively neglected area of research in the tropics. If the ie nature of the problem of post-harvest losses is to be fully understood and appropriate remedial measures introduced in veloping tropical countries, it is vital that this important area of research be given higher priority in future fisheries development ogrammes.

eferences

nggawati, A.M. et al. 1986. Seasonal abundance of flies and bacteria at two fish landing sites in West Java. Unpublished report of Research Institute of Fish Technology, Jakarta, Indonesia.

sastyasih, M. and Madden, J. 1986. Effect of plant products and extracts on the acceptability of fish to flies. Unpublished report of Research Institute of Fish Technology, Jakarta, Indonesia.

on-Pedro, K.N. 1985. Toxicity of some citrus peels to *D. maculatus* Deg. and *C. maculatus* (F.). J. Stored Prod. Res., 21 (1): 31-34.

ser, J.R., Hanson, S.W. and Taylor, K.D.A. 1985. Loss reduction techniques for salted/dried marine catfish (Arius sp), In: Spoilage of Tropical Fish and Product Development., A. Reilly (ed.) Proceedings of a Symposium held in conjunction

with the Sixth session of the Indo-Pacific Fishery Commission Working Party on fish technology and marketing. Royal Melbourne Institute of Technology, Melbourne, Australia, 23- 26 October 1984. FAO Fish Rep., (317) Suppl.:297-305, 474 p.

Esser, J.R. et al. 1986. Evaluation of insecticides to protect salted-dried catfish from insect infestation during processing and storage. In: Multi-Disciplinary Studies on Fisheries and Inshore Coastal Resource Management, Proceedings of a Seminar Organised by Diponegoro University, Semarang, Bandung Institute of Technology and Newcastle University. Semarang, Indonesia, 21-26 July 1986. 1:181-215. 267 p.

Esser, J.R. 1988. Effect of salt concentration on oviposition and larval growth and mortality in blowfly Chrysomya megacephala (Fab.). R. Sunness (ed.), Proceedings of a Symposium held in conjunction with the seventh session of the Indo-Pacific Fishery Commission Working Party on fish technology and marketing. Food and Agriculture Organisation Regional Office, Bangkok, Thailand, 19-22 April 1988.

Esser, J.R. et al. 1988. Prevention of insect infestation and losses of salted-dried fish in Indonesia by treatment with an insecticide approved for use on fish. R. Sunness (ed.), Proceedings of a Symposium held in conjunction with the seventh session of the Indo-Pacific Fishery Commission Working Party on fish technology and marketing. Food and Agriculture Organisation Regional Office, Bangkok, Thailand, 19-22 April 1988.

FAO 1981. The prevention of losses in cured fish. FAO Fisheries Technical Paper. No. 219: 87 p.

Gordon, A.E. and Esser, J.R. 1989. Post-harvest losses of fish in Indonesia. Report 7. Financial evaluation and adoption of the insecticide pirimiphos-methyl (Minawet) as a protectant of cured fish against blowfly infestation. A report of field investigations conducted in Indonesia during May-June 1989. Overseas Development Administration, London and Directorate-General of Fisheries, Jakarta.

Hodges, R.J. 1984. Biological methods for integrated control of insects and mites in tropical stored products. II The use of pheromones. Trop. Stored Prod. Info., 48:9-15.

Lawson, J.R. and Gemmell, M.A. 1985. The potential role of blowflies in the transmission of taeniid tapeworm eggs. Parasitology, 91:129-143.

Pillai, P.R.P. 1957. Pests of stored fish and prawns. Bull. Central Research Institute, Univ. Kerala, Trivandrum, India. Series C 5 (3):79 p.

Poulter, R.G., Ames, G.R. and Evans, N.J. 1988. Post-harvest losses in traditionally processed fish products in less developed countries. In: Post-harvest Fishery Losses, M.T. Morrissey (ed.), Proceedings of an International Workshop held April 12-16, 1987, at the Univeristy of Rhode Island, Kingston, RI. ICMRD, 1988. 133-145, 250 p.

Rattagool, P., Metathip, P. and Esser, J.R. 1988. Evaluation of insecticides to protect salted-dried marine fish from insect infestation during processing and storage in Thailand. R. Sunness (ed.), Proceedings of a Symposium held in conjunction with the seventh session of the Indo-Pacific Fishery Commission Working Party on fish technology and marketing. Food and Agriculture Organisation Regional Office, Bangkok, Thailand, 19-22 April 1988.

Rollings, M.J. and Hayward, L.A.W. 1963. Aspects of the dried fish trade in Nigeria with particular reference to Lake Chad. Trop. Stored Prod. Inf., 5:162-167.

Walker, D.J. and Evans, N.J. 1984. Report on two visits to the Gambia to evaluate the use of insecticides in reducing losses in traditionally cured fish. Unpublished report R1245(S) of Tropical Development and Research Institute, London, UK.

Whitle, K.J. 1985. A vital role for aquatic resources in feeding a hungry world. Proceedings of the Nutrition Society. 44:19- 29.

ELABORATION OF A RESEARCH METHODOLOGY FOR IMPROVING THE QUALITY OF DRIED FISH IN MALI

Nadine Zakhia, Jean-Louis Cuq** and André Themelin**

*Centre d'Etudes et d'Expérimentation en Mécanisation Agricole et Technologie Alimentaire, 73 rue J.F. Breton, 34000 Monpellier, France.

**Centre de Génie et Technologie Alimentaires, Université des Sciences et Techniques du Languedoc, Place Eugène Bataillon, 34100 Montpellier, France.

Summary

Processed fish in developing countries is often of a poor and irregular quality. Our research and development programme aims at improving the quality of dried fish in Mali (West Africa), by controlling the parameters of the drying process. This paper deals with the elaboration of a work methodology appropriate to the Malian context, i.e. seasonal fluctuations, complexity of the marketing flows, specific problems of cured fish storage. This methodology consists of defining a "standard Malian dried fish" (for one species) according to the local tastes and food habits of Malian consumers. The characterisation of this "standard" by "scientific criteria" will point out the impact of drying parameters on raw fish and will permit an optimisation of the drying process. The results obtained should enable us to propose recommendations for technological improvements and standards for a better quality control of dried fish.

Introduction

The study we present in this paper is a part of a multidisciplinary programme entitled: "Improvement and Control of the Quality of Fish Processed in the Continental Tropics". Several organisations participate in this project, according to their expertise and relations. These partners are: CEEMAT/CIRAD (France); MUCL (Belgium); ISFRA and OPM (Mali); LMUB (Burundi) and FAT (Thailand) (see abbreviations at end of paper). The programme mainly aims at:

- reducing fish post harvest losses in Mali, Burundi and Thailand by improving fish processing and storage
- defining adequate and specific quality criteria so as to evaluate cured fish products
- increasing the value added to cured fish so that small-scale fishery and traditional fish processing may become more dynamic

In developing countries, traditional fish processing cannot guarantee absolute protection nor regular quality of the final products. This lack of quality has severe consequences upon commercial dealings, whether regional, national or for export. Besides, quality assessment of fish (raw or cured) in the tropics is very limited and non-comparative, because of the use of general quality criteria, unadapted to the local context. Because of these problems, this research-development project aims to propose solutions which take into account the natural environments of the countries concerned.

The first application concerns Mali. This paper deals with the quality control of Malian dried fish. First, we present our preliminary trials and the problems we encountered; then, we explain the work methodology chosen for the following stages of our study.

Survey of Malian fishery

Mali is a West African continental country crossed by the Niger river, which has its source in Guinea and passes successively through Mali, Niger and Nigeria. In Mali, the river branches out, forming an inner delta called the Niger Central Delta where the Malian fishery is most active (INRZFH and ORSTOM, 1988).

Before the drought of 1970-1973 fish catches reached 80,000 to 100,000 tons per year. Since the drought of 1980-1983 catches do not exceed 60,000 tons per year (Marc, 1989; Moustaid, 1989). Fish is a very important foodstuff in Mali, providing a third of the total animal proteins consumed. The average fish consumption of Mali is 2.2 kg of fresh fish and 6.8 kg of cured fish per capita per year (TDRI and CEASM, 1986).

The Malian fishery is exclusively small-scale. It employs 100,000 to 150,000 workers, including fishermen and agents involved in subsidiary activities. Fish is caught either near the river bank or further out by using canoes and small boats. The main fishing gears used in Mali are hook and line, tangle-nets, draw-nets, cast-nets and baskets. In addition, the large variability of water levels in the Niger river induces four distinct fishing seasons during the year:

- July to September, start of the river's rising, very little fishing activity
- October to November, river in full spate, reduced fishing activity
- November to February, start of the river's subsidence, start of active fishing season
- March to June, full subsidence of the river, very high fishing activity

The fishermen may be either professional Malians (Bozo and Somono ethnic groups) or professional foreigners coming from Niger (country). They also may be semi-professionals or temporary fishermen from the Bambara, Sonrai, Marka and to a lesser extent Peul ethnic groups (INRZFH and ORSTOM, 1988; Themelin and Gerbe, 1987).

Only fishermen near towns and urban centers can sell fresh fish; otherwise, most cured fish products mainly concern migrant fishermen who move (with their families) along the river and set up temporary fishing camps. Fishing is a family business and work is divided as follows: men catch the fish and women are responsible for processing fish and selling it, either to middlemen or directly on small local markets.

Traditional fish drying in Mali consists of removing fish heads, gills and guts, splitting big fish into two and washing them in the river. Fish are often left in the water for a night so as to undergo fermentation. Afterwards, they are put to dry in the sun, either on the ground (directly or on straw mattings) or on elevated racks. Sundrying lasts two days for small fish, four days for medium fish and around ten days for bigger ones. These drying conditions obviously lead to much deterioration of the fish and to their contamination by wind, dust and insect or animal attacks, resulting in a very poor and irregular quality.

This survey points out the complexity of our study because of the large natural fluctuation of fish during the year. Besides, due to the variations of water levels in the Niger river, fish move so as to find an adequate environment for their nutrition and reproduction. Consequently, migrant fishermen "follow" the fish and move along the river. We therefore have to deal with these two aspects of the fishery in Niger Central Delta; the ichthyological changes (fish moving) and the human movement (fishermen scattering).

Preliminary trials on Malian fish

The fish trials we carried out consisted of drying three Malian fish species under different conditions. The species tested were *Tilapia monodi* (lean fish), *Labeo coubie* (lean fish) and *Hydrocynus brevis* (fatty fish). Experimental conditions are represented by the following combinations:

1. T = 60°C; H = 60%; v = 2 m/s
2. T = 60°C; H = 10%; v = 2 m/s
3. T = 40°C; H = 60%; v = 2 m/s
4. T = 40°C; H = 10%; v = 2 m/s

where T, H and v are the drying air parameters (T = temperature; H = hygrometry or relative humidity and v = velocity).

The frozen fish was received from Mali. The heads were removed and the fish was split through the backbone. One half remained frozen under vacuum for later analysis. The other half was set to thaw at 4°C overnight; then it was scaled, degutted, washed and cut into 1 cm wide strips. These strips were set to dry in a thin layer in a hot air stream. A weighing machine, connected

to the drying chamber allowed the weight to be followed during drying. Finally, dried fish and "fresh" fish (i.e. the first half of the fish kept stored as frozen and thawed before analysis) were subjected to analysis (Table 1).

Some general conclusions may be drawn (Marc, 1989; Moustaid, 1989):

- drying at 40°C gives a harder texture, especially for lean fish
- the drying process seems not to alter the fish protein quality
- the relative humidity 60% might be interesting for an eventual recycling of drying air
- in fatty fish, lipids seem to slow down the water evaporation

These conclusions only allow for a general assessment of our trials and should be cautiously interpreted. This study pointed out many problems and gaps that were to be avoided afterwards, such as:

- non-controlling of the fish history (before, during and after the catch)
- lack of repeatability of analyses
- deficiency of raw material and supply delays
- sampling heterogeneity (both drying and analysis sampling)
- ignorance of criteria for the assessment of dried fish quality specific to Mali

Elaboration of the work methodology

The methodology aims at planning the further stages of our study, taking into account the problems and gaps cited above. However, this methodology is only a working basis; it is obvious that many changes may be needed according to local conditions. The methodology consists of two working phases: one in Mali and the other in France. Depending on financial aid availability and fishing seasons in Mali, the phases may or may not occur together.

Phase in Mali

It may be divided into three sections namely:

1. Thorough study of the Malian fishery. Definition of traditional drying parameters:

   a. accompanying a fisherman noting all fishing details (season, species, gear, handling, fish trimming before drying, insecticide treatments, ...)

   b. following of traditional drying process by simple on-the-spot measurements (air temperature, relative humidity, velocity, decrease in fish weight and moisture content determination, drying duration)

   c. fish packaging and storage after drying, means of transport to the market

2. Assessment of dried fish quality on Malian criteria. Study of dried fish marketing:

   a. investigations on markets (fish stalls, species exposed); inquiries among fish processors on fish selling and buying criteria of dealers and consumers according to the dried fish dishes to be prepared. These inquiries will not be complex questionnaires but simple dialogues with the concerned agents

   b. elaboration of 3 tasting panels, representing housekeepers, dealers and processors. These panels will be asked to assess the quality of dried fish sampled at fishing camps or market. The panel will be free to choose the taste description they find suitable to the Malian context without any influence on vocabulary

3. Definition of a Malian standard dried fish with strict control of sample homogeneity:

a. first we have to contact a fisherman interested in our campaign; then we will accompany him on boar equipped with ice and clean containers

b. as fishing conditions (fish species and size, fishing gear) will not differ during the trip, the catch l will be homogeneous. As soon as the fish is caught, a sample of 3 fishes will be taken from this l and covered with a thick layer of ice for later analysis. This sample will be the reference for comparis with the dried fish. The remaining fish will then be handled by the fisherman as usual. All fishin details will be noted

c. during unloading, after fermentation, trimming and at different stages a sample of 3 fish will be take from the same batch (Figure 1). All climatic variations (sudden changes of temperature, hygrometr wind, clouds, ...) will be noted

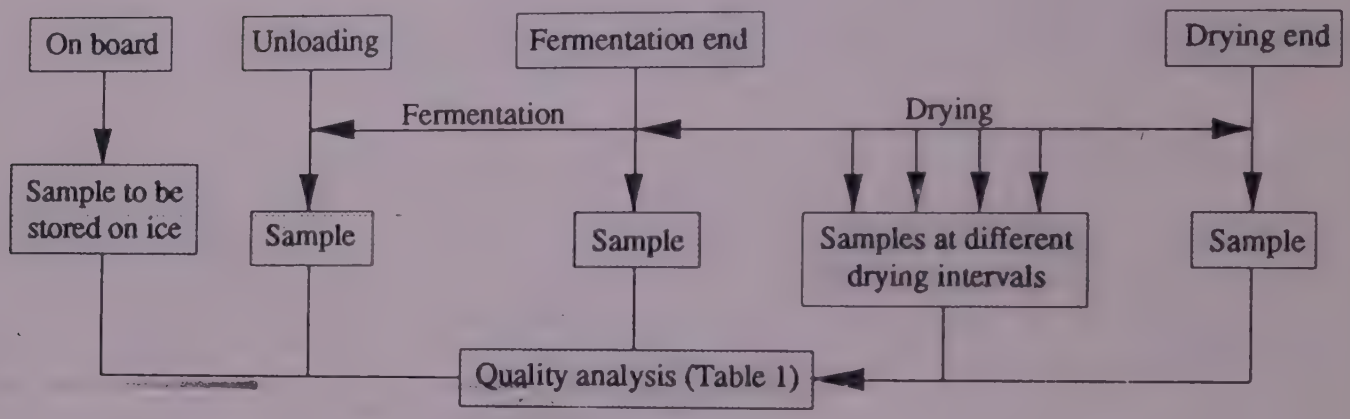

Figure 1. Fish sampling in Mali phase

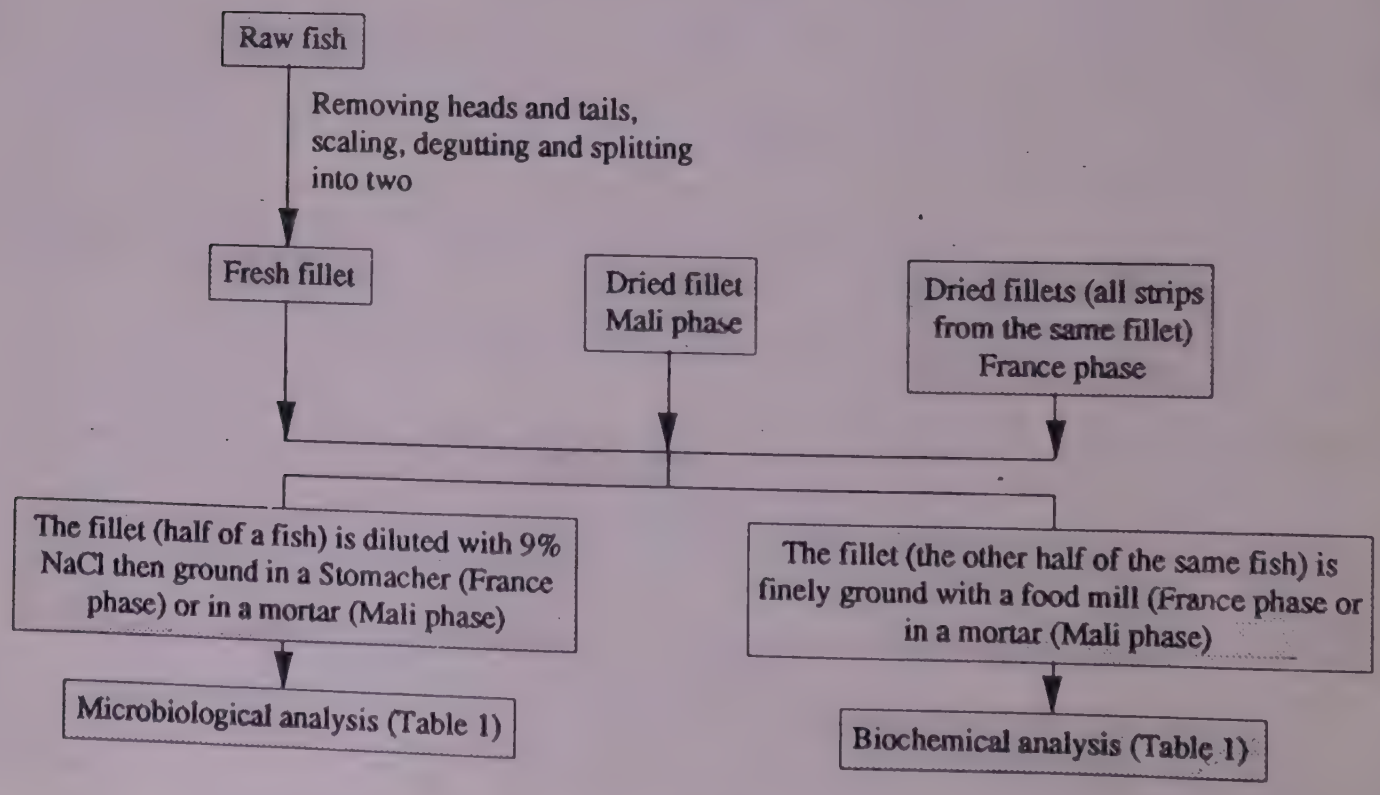

Figure 2. Fish trimmings for analysis

All fish samples will be subjected to simple on-the-spot analysis (Table 1). Fish trimmings for analysis are given in Figure 2.

Table 1. Quality analysis for both raw and dried fish samples

	Preliminary trials	Further trials	
		France phase	Mali phase
Microbiological analysis			
Total aerobic mesophilic flora	+	+	+
Total and faecal coliforms	+	+	+
Yeasts and moulds	+	+	+
Salmonella	+	+	+
Anaerobic sulfate reducers	-	+	+/-
Biochemical analysis			
General			
Moisture content	+	+	+
Proteins			
Total basic volatile nitrogen	+	+	+
Trimethylamine	+	-	-
TNBS (trinitrobenzene sulfonic acid) index	+	+/-	-
Total amino acids	+	+	-
Free amino acids	+	-	-
In vitro digestibility	+	+/-	-
Lipids			
Total lipids	+	+	-
Iodine value	+	-	-
Peroxide value	+	+	-
Thiobarbituric acid value	+	+/-	-
Anisidine index	-	To be tried	+/-
Ultra violet absorbance value	-	To be tried	+/-
Nucleotide catabolism			
Hypoxanthine value	+	+	+/-
Sorption isotherms	+	+/-	-
Texture measurements	+	+	-
Colour measurements	+	+	-
pH value	+	+	+/-
Sensory evaluation (with tasting panels)	+	-	++

NB: (+) Adopted test; (-) non adopted test; (+/-) to be done only if necessary

Phase in France

Obtaining fish from Mali presents serious problems (delivery delays, controlling of fish history). To avoid these, we will dry either French freshwater fish or tropical ones available in France. These species should be as similar as possible to the Malian

ones in terms of composition. Because of the great diversity of fish species in the Niger river, it would be interesting to carry out this study on two species, a lean and a fatty one. Fish behaviour under drying conditions will be followed by analysis (Table 1).

We have already contacted CEREMHER, near Montpellier (France) where *Tilapia niloticus* is farmed (a species commonly caught and consumed in Mali). We will then be regularly supplied with raw fish. In the same way, we will be able to control homogeneity (size, weight, feeding).

Drying experiments will be carried out using the CEEMAT drying chamber in which convective hot air passes through a thin layer of product. However, the dimension of drying racks does not allow fish to be split as is traditional in Mali. So we will cut the fish through the backbone into 1 cm thick strips which will be labelled. All strips from the same fillet will be mixed for analysis (Figure 2). Samples of 3 fish will be taken from the lot before and after drying (5-10% moisture). These samples will undergo analysis as shown in Table 1. Fish trimmings for analysis are given in Figure 2. Triplicate analysis will permit statistical treatment.

Steps already completed

Within the framework described, we first produced an experimental design for subsequent drying trials. Next we carried out a comparative study of classical microbiological methods with rapid kits.

Design of drying experiments

Our main aim is to assess the impact of drying parameters on fish quality. Good experimental design allows this to be investigated with fewer experiments. Quantitative data obtained from the experimental design may be associated with a statistical method called Response Surface Methodology (RSM) so as to optimise the drying process according to the measured response. Experimental design is generally based on a second order mathematical model (Giovanni, 1983; Thompson, 1982). For our study, a central composite rotatable design was adopted. It consists of:

- 3 input factors (or drying parameters): temperature T (°C); hygrometry H (%) and air velocity v (m/s)
- 5 levels of each of the above three factors were chosen. A value of -1.682 was assigned to the lowest level; 0 to the middle level and +1.682 to the highest level. Values between -1.682 and +1.682 for the three factors were determined by calculation
- Measured responses (or effects of the different factors on the product). In our case, measured responses are the quality analysis of dried fish (Table 1; phase in France).

Our experimental design may then be summarised in 20 drying combinations (including 6 replicates of the central point) and may be represented by the following matrix (Table 2).

Table 2. Drying combinations using temperature, humidity and air velocity

Experiment	T(°C)	H(%)	v(m/s)	Experiment	T(°C)	H(%)	v(m/s)
1	35	25	1	11	50	50	1.5
2	65	25	1	12	50	50	1.5
3	35	75	1	13	50	50	1.5
4	65	75	1	14	50	50	1.5
5	35	25	2	15	25	50	1.5
6	65	25	2	16	75	50	1.5
7	35	75	2	17	50	8	1.5
8	65	75	2	18	50	92	1.5
9	50	50	1.5	19	50	50	0.66
10	50	50	1.5	20	50	50	2.34

To avoid bias, the total of 20 runs will be performed in a random order. Other factors such as the fish species, fishing season, fish fillet thickness, fermentation (if systematic in Mali) are unvariable for commodity even if involved in the drying process.

After data collection, a statistical variance analysis will alow to estimate the model parameters. These parameters help in fitting data to all possible combinations of the terms in the model mathematical equation. The coefficient of multiple determination R2 indicates the correlation between measured responses and those predicted by the model. The most significant input factors are also evidenced. Finally, computer programme will be used to draw response contour plots representing the model equation.

Microbiological comparative study

Fish is highly perishable and may be contaminated during processing, so it is essential to strictly control its bacteriological quality. Nevertheless, classical microbiological methods are demanding. Some rapid methods (or kits) are now available for the detection and/or counting of specific bacteria. These tests are ready-to-use and merit testing for on-the-spot application in Mali. For this reason, we compared some of these kits with classical methods so as to estimate their selectivity (Table 3).

In classical methods, culture media were prepared and poured into Petri dishes, except for liver veal agar available in ready-to-use tubes (diagnostic Pasteur Co.). Rapid kits were simply used according to instructions allowing great time savings.

Table 3. Comparison of classical methods and rapid kits

	Classical Methods	Rapid kits	Selected methods for future use
Total aerobic mesophilic flora	Plate Count Agar (PCA)	• Millipore • Petrifilm • Microtest A	• Millipore • Petrifilm
Coliforms	• Desoxycholate Lactose Agar (DCL) • Violet Red Bile Agar (VRBA)	• Millipore • Petrifilm	• Millipore • Petrifilm
Yeasts	• Potato Dextrose Agar (PDA)	• Millipore • Microtest A	• Classical PDA for counting • Millipore for global evaluation
Salmonella	• Pre-enrichment - Buffered Peptone Water • Enrichment - Selenite Cystine Broth • Post enrichment - M Broth • Plating - SS Agar	• Tecra test • Spectate test • 1-2 test (all these tests are detection ones)	• Tecra test
Anaerobic sulfate reducing bacteria	• Liver Veal Agar (LV tubes ready to use)	• Microtest SR	• LV tubes ready to use

For this preliminary comparative study microbial cultures were used as inoculum instead of fish. The strains used were *Escherichia coli*, *Enterobacter aerogenes* and *Citrobacter* for coliform group; *Saccharomyces cerevisiae* CBS 1512 for yeast group; *Salmonella typhimurium* IP 5858 for *Salmonella* group; *Clostridium sporogenes* for anaerobic sulfate reducing bacteria; *Proteus mirabilis*, *Micrococcus luteus*, *Staphylococcus aureus* IP 483 and *Streptococcus faecalis* IP 53152 (these last four bacteria were only used in association with the ones cited above so as to form the total microbial flora). For each bacterial group (i.e. total flora, coliforms, yeasts, sulfate reducers and *Salmonella*), tests were carried out on both "exponential" and "exponential + 3 days" microbial cultures (Table 4). Each of the classical and rapid methods were triplicated to further statistical analysis. We also systematically prepared a positive reference (only one pure microbial strain X); total flora (all bacteria including the strain X) and a negative reference (the total flora without the strain X) (Table 4).

Table 4. Inoculum preparation for each bacteria group analysis

	Positive reference	Total flora	Negative reference
Exponential culture	Strain incubation in nutrient broth at 37°C for 24 h (except for *Clostridium* incubated in Rosenow medium)		
Exponential + 3 days culture	After the exponential phase, keeping strains at 20°C for 3 days		

The principle of rapid kits is described as follows:

- Petrifilm (3M Biomedical CO.): It consists in a dry rehydratable film where the nutrients are embedded. One ml of liquid sample (microbial dilutions) is added directly to the self-contained culture plate, then it is spreadout with a plastic diffusor and incubated at 37°C (24 h for coliforms and 48 h for total flora). Viable colonies are counted; specific coliform colonies are surrounded with bubbles).

- Millipore (Millipore Co.): This sampler is constructed to combine both an intimate contact of a 0.45 µm membrane filter to a nutrient-pad and the incorporation of an air-vent on the upper back portion of the paddle. This configuration allows for the draw-through of 1 ml of sample to affix microorganisms to the filter surface for subsequent culturing within its transparent plastic case. (Incubation at 37°C, 24 h for coliforms, 48 h for total flora and 72 h for yeasts and moulds). The filter is grid-marked to aid in counting the microbial colonies.

- Microtest A (France Organo Chimique Co.): Nutrients embedded in this two-sided plate allow for counting of total flora (plate count agar on light side, incubation at 37°C for 48 h) and yeasts and moulds (malt agar on dark side, incubation at 37°C for 72 h).

- Microtest SR (France Organo Chimique Co.): This system consists in drawing liquid sample through a capillary, then introducing it into a sterile tube containing appropriate nutrient agar (with a sulfate reducer). The inoculate tube is incubated at 37°C for 48 h.

- Tecra test (3M Sante Co.): is an immunoenzymatic test. The already enriched sample is heated at 100°C for 15 min., then introduced into holes containing specific antibodies. *Salmonella* is detected by green colouring within 2 h.

- Spectate test (Rhone Poulenc Co.): *Salmonella* is detected within 4 min. by the clumping of latex beads already sensitised with several specific antibodies. The colour may differ according to the different *Salmonella* groups and is compared to a reference scale.

- 1-2 Test (Biocontrol Co.): is a rapid qualitative method for the detection of motile *Salmonella*. The sampler is a plastic device with two chambers containing nutrients, interconnected by a small opening. The sample is inoculated in the first chamber. If *Salmonella* are present, they move through the second chamber and are stopped by flagellar antibodies, then developing a visually readable band of cells within 24 h.

Comparisons of classical methods and rapid tests were statistically analysed. This allowed us to select several kits useful for microbiological control in Mali (Table 3). These selected kits will need to be adapted (in terms of appropriate dilutions) to dried or fresh fish analysis.

References

Giovanni, M. 1983. Response surface methodology and product optimisation. Fd. Technol., November, 41-45, 83.

INRZFH and ORSTOM. 1988. Etudes halieutiques du delta central du Niger. Rapport d'enquêtes au Mali.

Marc, C. 1989. Influence des variables du procédé de séchage sur l'évolution de certains critères de qualité de trois espèces de poisson du fleuve Niger au Mali: analyse particulière du comportement des lipides. Rapport de mastère en technologie agro-alimentaire pour les régions chaudes. ENSIA/SIARC, Montpellier, 171 p.

Moustaid, F. 1989. Influence des variables du procédé de séchage sur l'évolution de certains critères de qualité de trois espèces de poisson du fleuve Niger au Mali: analyse particulière du comportement des protéines constitutives. Rapport de mastère en technologie agro-alimentaire pour les régions chaudes. ENSIA/SIARC, Montpellier. 158 p.

Themelin, A. and Gerbe, A. 1987. Elaboration d'un programme national concerté sur l'amélioration des techniques de conservation du poisson par séchage solaire au Mali. Rapport de mission. Agence de Coopération Culturelle et Technique, 32 p.

Thompson, D. 1982. Response surface experimentation. J. Fd. Processing and Preservation, 6, 155-188.

TDRI and CEASM, 1986. Evaluation des pertes de poisson en Afrique de l'Ouest. Rapport élaboré sous l'égide du CTA. Ede-Wageningen, The Netherlands, 92 p.

Abbreviations

CEEMAT	Centre d'Etudes et d'Expérimentation en Mécanisation Agricole et Technologie Alimentaire, département du
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement (France)
MUCL	Mycothèque de l'Université Catholique de Louvain (Belgique)
ISFRA	Institut Supérieur de Formation et Recherche Appliquée (Mali)
OPM	Opération Pêche Mopti (Mali)
LMUB	Laboratoire de Microbiologie de l'Université du Burundi (Burundi)
FAT	Faculty of Agricultural Technology - King Mongut's Institute of Technology Ladkrabang (Thailand)
INRZFH	Institut National de Recherche Zootechnique, Forestière et Hydrobiologique (Mali)
ORSTOM	Institut National de Recherche Scientifique pour le Développement en Coopération
ENSIA	Ecole Nationale Supérieure des Industries Agricole et Alimentaires (France)
SIARC	Section Ingénieurs Industries Alimentaires Régions Chaudes de l'ENSIA (France)

PRODUCTION OF FISH HYDROLYSATE FROM *OREOCHROMIS MOSSAMBICUS* AND ITS APPLICATION IN FISH CRACKERS (*KEROPOK*)

S.Y. Yu and L.K. Tan
Faculty of Food Science and Biotechnology University, Pertanian Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Summary

To improve the flavour of *Oreochromis mossambicus*, its flesh was hydrolysed using the enzyme alcalase 0.6 L. The hydrolysis was carried out at 50°C, using a ratio of 1 part water and 1 part fish mince, an enzyme: substrate ratio of 2% at pH 8.0. The reaction was terminated by heating to 90°C for 20 min. and the solution was neutralised. The soluble fraction obtained after centrifugation was spray-dried in a mini spray dryer at an air inlet temperature of 170°C and a feed rate of 4 L/h. An acceptable hydrolysate was obtained after 4.5 h of hydrolysis. This corresponded to a degree of hydrolysis of 12.5%. The spray-dried hydrolysate was incorporated into fish crackers and a 10% hydrolysate was found to give maximum linear expansion. Sensory evaluation with 20 experienced panelists showed that in terms of appearance, crispiness and colour, fish crackers with hydrolysate had the highest scores, compared to crackers made from *O. mossambicus* and *Sciaena spp.* There was no significant differences in overall acceptability in all three samples. Crackers with hydrolysate also had the highest protein and lowest fat contents. The hydrolysate was analysed for proximate composition and amino acid content.

Introduction

Oreochromis mossambicus is becoming one of the most widely farmed fish in Asian aquaculture. It is very adaptable to environmental changes, eats virtually everything and is a prolific breeder.

However, direct consumption of *O. mossambicus* in Malaysia is infrequent due to the presence of a muddy flavour associated with freshwater fish, and also the general unattractive external appearance of the species. One of the ways in which flavour and thus acceptability could be improved is by enzymic hydrolysis, in which proteins are broken down to produce polypeptides with improved flavour. The hydrolysate thus produced can then be applied to the appropriate food material to increase protein content and improve flavour.

This paper describes the hydrolysis of proteins from *O. mossambicus* using the enzyme alcalase and the application of the hydrolysate in fish crackers.

Materials and methods

Raw materials

Freshly caught *O. mossambicus* was obtained from the local market, kept in ice and deboned immediately upon arrival in the laboratories, using a locally fabricated deboner (Ban Hing Weights and Measures Co., Kuala Lumpur). The deboned flesh was then leached twice with twice its volume of water before mincing in a bowl cutter (ADE SL18, Hamburg). Alcalase 0.6 L, a commercial protease in which active enzyme is *Subtilisin Carlsberg*, was a gift from Novo Industry A/S, Denmark.

Hydrolysis reaction

The hydrolysis reaction was carried out as in Figure 1. The mixture maintained at pH 8.0 by the addition of 4N NaOH. The degree of hydrolysis (DH) was calculated using the method of Alder-Nissen (1979). The neutralised-soluble fraction was spray-dried in a Buchi 190 spray-dryer (Switzerland) at an air inlet temperature of 170°C and an outlet temperature of 90°C. The feed rate was 4 L/h.

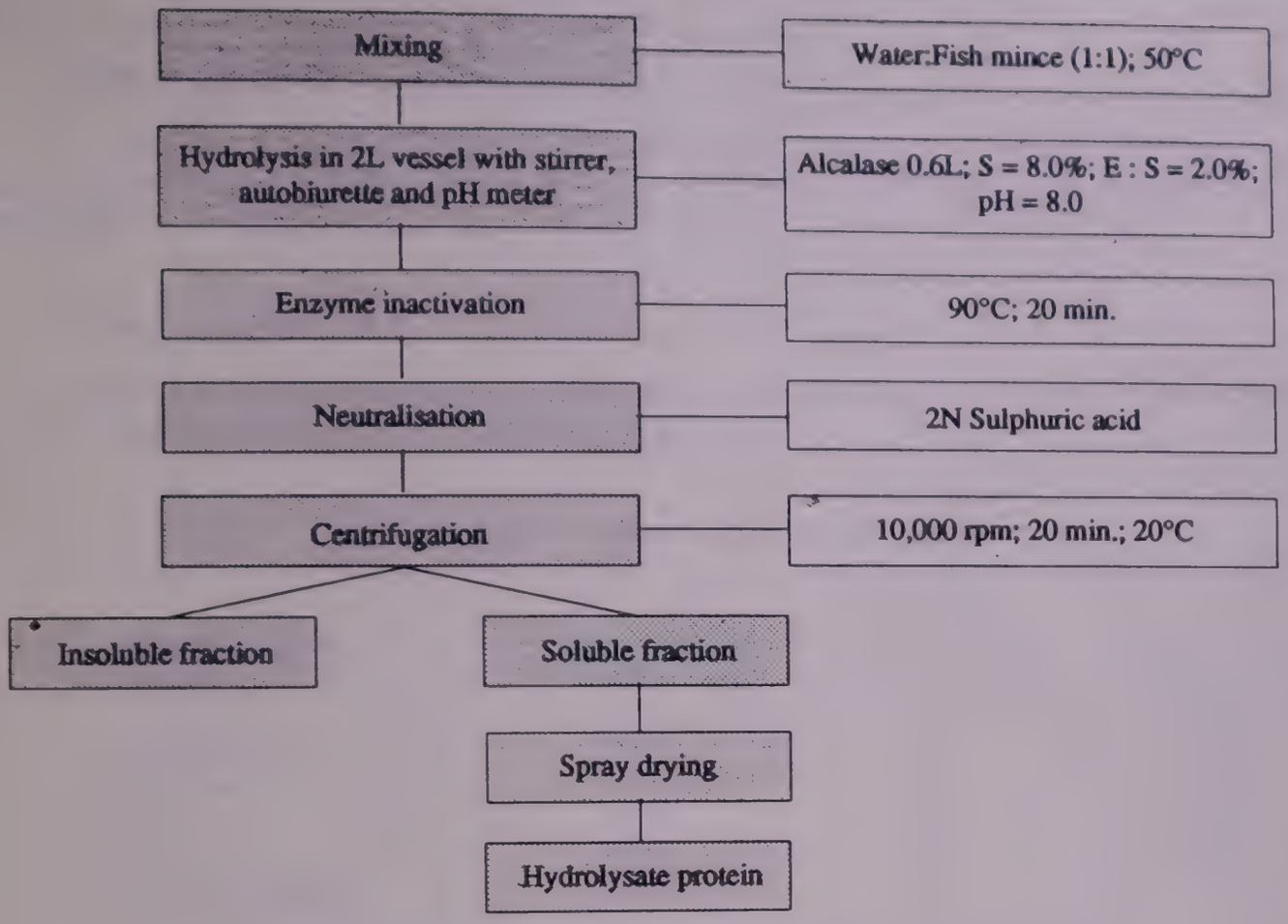

Figure 1. Production of enzymatic fish protein hydrolysate from *Oreochromis mossambicus*

Preparation of the fish crackers

The crackers were prepared according to the method of Siaw et al. (1985).

Linear expansion

The percentage linear expansion was calculated as in Siaw et al. (1985).

Sensory evaluation

Twenty experienced panelists were chosen to evaluate the product using a Hedonic scale of 9 for 'like very much' and core of 1 for 'dislike very much'. Analysis of variance of the sensory data determined significant differences among the samples armond, 1977).

Proximate analysis

Protein, fat, moisture and ash contents were measured according to the AOAC method (1975).

Amino acid analysis

Free amino acids were determined using an amino acid analyser (Technicon TSM, USA) after treating the hydrolysate ith 6N HCl at 110°C for 24 h.

Results

Degree of hydrolysis

The optimum reaction time for an enzyme:substrate ratio of 1:50 was 5 h. The degree of hydrolysis was 12.5%. At 4-4.5 h the hydrolysate was acceptable. Panelists detected bitterness after 5 h of hydrolysis. The hydrolysate obtained after 4.5 h of hydrolysis was used.

Sensory evaluation

Table 1 shows that in terms of appearance, crispiness and colour, crackers with hydrolysate had the higher scores, higher compared to crackers made with *O. mossambicus* and *Sciaena spp.*

Table 1. Sensory evaluation of crackers

Organoleptic qualities of crackers	Mean score		
	Hydrolysate crackers	*Oreochromis* crackers	*Sciaena* crackers
Appearance	6.67 a	4.56 b	5.50 b
Crispiness	7.28 a	5.83 b	6.11 b
Colour	6.61 a	4.83 b	4.72 b
Flavour	4.56 b	4.78 b	6.17 a
Overall acceptability	5.22 a	4.88 a	5.83 a

Means followed by the same letter are not significantly different at the 5% level

Crispiness is the most important parameter for fish crackers and has the least degree of tolerance. Crispiness can also be measured as percentage linear expansion. Table 2 shows that incorporation of 10% hydrolysate resulted in maximum linear expansion.

Table 2. Effect of hydrolysate on the linear expansion of crackers

% Hydrolysate	% Linear expansion
0.0	95.40
2.5	94.78
5.0	99.13
10.0	113.90
15.0	102.72
20.0	100.29
25.0	89.90

The colour of hydrolysate crackers attracted the highest scores among the three samples (Table 1). This is mainly due to the loss of the dark discolouration which was centrifuged out after hydrolysis.

Proximate analysis

The proximate composition of the spray-dried hydrolysate is shown in Table 3.

Table 3. Proximate composition (%) of the spray-dried hydolysate

	Protein	Ash	Moisture	Fat
Spray-dried hydrolysate	90.10	6.68	1.80	2.13

Among the three samples tested, crackers with hydrolysate had the highest protein and lowest fat contents, (Table 4) thus nutritional value as a snack food is improved.

Table 4. Protein and fat content of crackers

	% Protein	% Fat
Hydrolysate crackers	25.84	0.48
Oreochromis crackers	16.23	0.93
Sciaena crackers	16.74	1.40

Table 5. Amino acid (mg/g) composition of *O. mossambicus* and its hydrolysate

Amino acid	*O. mossambicus* (mg/g)	Hydolysate
Alanine	64.25	58.17
Arginine	68.19	60.23
Aspartic acid	86.87	87.37
Cystine	10.93	10.61
Glutamic acid	148.81	134.81
Glysine	58.03	46.05
Histidine	26.58	28.61
Isoleucine	38.04	38.44
Leucine	73.45	60.89
Lysine	118.96	127.76
Methionine	20.48	21.36
Phenylalanine	30.69	32.22
Proline	26.32	22.63
Serine	34.43	37.24
Threonine	36.98	37.87
Tyrosine	24.53	27.89
Valine	47.76	36.49
Total essential amino acid	461.13	443.87
Total amino acid	915.30	858.64

Analyses of the hydrolysate

A total of 17 amino acids were identified from the proteins of *O. mossambicus* and its hydrolysate (Table 5). The hydrolysate basically retained the same amino acid profile of *O. mossambicus*, thus confirming its potential as a good protein source.

Acknowledgement

The authors wish to thank IFS for providing financial assistance for this project.

References

Adler-Nissen, J. 1979. Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzene sulfonic acid. J. Agric. Fd. Chem., 27:1256-1262.

AOAC 1975. Official Methods of Analysis, Association of Official Chemists, William Horwitz (ed.), 305 AOAC, Washington, D.C.

Larmond, E. 1977. Laboratory Methods for Sensory Evaluation of Food., Research branch, Canada Department of Agriculture, Ottawa, Publication 1637.

Siaw, C. L., Idrus, A. Z. and Yu, S.Y. 1985. Intermediate technology for fish cracker (Keropok) production. J. Fd. Technol., 20, 17-21.

A STUDY ON ISOLATION OF SQUALENE FROM ACID ENSILAGED SHARK LIVER OIL

Sumpeno Putro and Sutijana***
*Technical Advisor, FAO Project RAS/84/027, INFOFISH, Kuala Lumpur, Malaysia
**Faculty of Mathematics and Natural Sciences University of Indonesia, Jakarta, Indonesia

Introduction

The shark is potentially valuable source of protein for domestic consumption as well as hard currency earnings for exports. It is, unfortunately, a resource that is frequently either not fully utilised or wasted.

While in principle every part of most sharks can be used for various processing, this is sometimes difficult to achieve on an industrial scale because of size and other biological features. Studies indicate that shark meat is suitable for dry salting, boiled salting or production of minced fish as raw material for fish jelly products such as fish bali and fish sausage. Shark skin, with proper tanning, can be converted into a high quality leather. Fins of the right size will always find a market, whereas the teeth could be used for ornamental products. The value of shark livers is steadily increasing, especially for those with squalene content of more than 80%.

Shark landings in Indonesia have steadily increased in recent years. Sharks are usually caught as by-catch of tuna long-lining, but since 1986, fishermen have begun concentrating on catching shark. This is mainly due to the increasing demand of shark liver oil and squalene for export. Among the important species of shark caught in the Indonesian waters are: hammerhead shark (*Sphyrna zygaena*), blacktip shark (*Carcharhinus limbatus*), porbeagle shark (*Lamna nasus*), and bottle shark (*Centrophorus squamosus*).

In Indonesia, the sharks are mostly processed into dry salted or boiled salted product popularly known as "pindang", and the fins are sundried which fetch high prices in many restaurants. In recent years, there have been efforts to maximise the use of liver oil as an important source of squalene for the cosmetics and pharmaceutical industries.

Scientific background

Research on the use of shark liver oil as raw material for pharmaceutical products has been carried out and is still continuing in many countries. Although vitamin A from shark liver oil has been widely replaced by synthetic vitamin A, the former is still being used in several countries, and more may follow in the future, if there is sufficient supply of high potency livers. Species from which liver oil for medical use (vitamins A and D) may be obtained are the black shark (*Galeus glaucus*), the mako shark (*Isurus glaucus*), the smooth-hound (*Mustelus manazo*) and hammerhead shark (*Sphyrna sp.*) (Kreuzer and Ahmed, 1978).

Shark liver oil is also the source of an acyclic hydrocarbons ($C_{30}H_{62}$) called squalene. Squalene is used in certain cosmetic preparations such as skin rejuvenators and for other purposes. Considerable interest on the use of squalene is steadily increasing in recent years, especially in Japan and Norway. Unfortunately, only a few species of shark have high squalene contents and considered to be economical for the extraction process.

Shark liver comprises between 10-25% of the total weight, which may contain about 60-75% oil (Broddy, 1965). Some ten shark species which belong to the family *Squalidae* contain liver oil bearing at least 80% unsaponifiable substances, most of it in the form of squalene (Kreuzer and Ahmed, 1978). The liver of dogfish (*Squalus acanthias*), greenland shark (*Sommiosus microcephalus*) and basking (*Cetorhinus maximus*) are usually big and with high oil content (Stansby, 1965). It is postulated that the oil content is very much influenced by several factors, including species, feed and environmental conditions. Shark inhabiting deep sea waters usually have a higher fat content (Mustafa, 1984). Table 1 shows the list of shark species with a high content of unsaponifiable substances.

Table 1. Content of unsaponifiable substances of selected shark species

Species	Unsaponifiable substance (%)
Squalus mitsukurina	87.32 - 90.17
Centrophorus squamosus	71.87 - 86.39
Centrophorus calceus	60.61 - 76.64
Centrophorus acus	62.90
Centrophorus atromarginatus	58.30
Cetorhinus maximus	22.80 - 55.30

Source: Kreuzer and Ahmed (1978)

Shark liver oil usually contains several compounds, especially triglycerides of various fatty acids, glycerol, glyceril phosphate, diacylglycerilether, cholesterol, vitamin A (retinol), vitamin D (calciferol), vitamin E (tocopherol), hydrocarbon compounds and pigments. Squalene belongs to hydrocarbon compounds and could serve as a precursor of cholesterol.

Squalene is difficult to detect if the content of unsaponifiable substances in the liver oil is less than 15% (Sunarya, 1987). This is mainly due to the fact that shark liver oils containing 16-28% of saponifiable substances contain only about 13.8% of squalene, whereas those with 60-90% contains about 84.4% of squalene. In addition to squalene, the hydrocarbon compounds comprise of pristane ($C_{18}H_{38}$) and zamene ($C_{18}H_{36}$) as well as other unidentified compounds, whereas in the Japanese Ishinagi shark (*Stereolepsis ishinagi*), it also contains another compound called gadusene ($C_{18}H_{32}$).

Shark liver oil can be extracted using different methods, including traditional sundrying, boiling, alkali digestion, rendering, biological ensiling and acid ensiling. Previous studies show that acid ensiling is the best method of oil extraction process, giving the highest yield and superior quality oil (Yunizal, 1983; Yunizal and Nasran, 1983; Abdurrahman and Saleh, 1976).

For these reasons, acid ensilaged shark liver oil is used in this study. The objective of the study is to improve the current practice of shark liver oil extraction in Indonesia as well as to find a suitable method for squalene isolation.

Materials and methods

Raw materials

Sharks obtained from Pelabuhan Ratu landing center, West Jawa, were caught by conventional long-lining. After weight and length measurements, the liver was removed and weighed. The liver was then washed and cut into pieces of about 1 cm^3 sizes.

Ensiling process

Shark liver cuts were then put into plastic bottles weighing 200 g each, followed by addition of 0.5 - 4% formic acid with 0.5% gradual increment in triplicates. The liver-acid mixture was mixed well and brought to the Research Institute of the Fish Technology, Jakarta, and incubated at room temperature in closed bottles for 4 days with thorough mixing once a day.

Oil separation

After the completion of the incubation period, the fermented liver-acid mixture was centrifuged at 2000 rpm for 10 min. The supernatant layer was then carefully decanted and put into a measuring flask containing about 10 g of anhydride sodium sulphate crystals and filtered. The oil filtrate was carefully stored in a closed bottle for further analysis.

Preliminary analysis of the oil

The acid and iodine values, and specific gravity were determined by the methods of the AOAC (1975); and the refractory index measured using a refractometer.

Isolation and purification of squalene

Five grams of liver oil was put into a 125 ml Erlenmeyer flask, and 3 ml of 28N potassium hydroxide solution and ethanol added followed by heating in a water bath for 30 min. with frequent mixing. The mixture was then allowed to cool to 40°C. Fifty ml of petroleum benzene, 20 ml ethanol and 40 ml aquadest were added and the mixture transferred into a separatory funnel. After rigorous mixing for about 10 min., the mixture was allowed to settle. The upper layer was transferred into another separatory funnel containing 20 ml of the aquadest while the bottom layer was re-extracted with 50 ml petroleum benzene. The upper layer was added onto the upper layer portion of the first extraction, while the bottom layer was discarded. This extract was then washed twice with 20 ml aquadest, followed by treatment with mild alkali and subsequent washing with 20 ml aquadest until an alkali-free extract was obtained. The extract was then transferred into a measuring flask containing 1.5 g of the anhydride sodium sulphate crystals and filtered using filter paper. The filtrate was put into a column chromatography containing fluorisil beads. The height of the column was 10 cm with a diameter of 1 cm. The eluent velocity was adjusted to about 1 ml/min. About 100 ml of eluent was collected for further analysis. The eluent was then evaporated in a vacuum chamber and the remaining solvent removed using nitrogen gas.

Qualitative analysis of the squalene extract

The specific gravity and refractory index of the squalene extract was assayed using the AOAC method. Squalene solubility was measured using petroleum benzene or n-hexane. The number of double bonds was qualitatively assayed by colourimetric method using a mixture of bromide and carbon tetrachloride solution. The squalene was also qualitatively identified on thin layer silica gel chromatography using n-hexane as eluent. The spots were then sprayed with sulphuric acid vapour.

Quantitative analysis of squalene

Quantitative analysis of squalene was carried out using gas chromatography as well as titrimetric method of AOAC.

Results and discussion

Acid ensiling

The results showed that the use of 1.5 - 2.5% formic acid gave the highest yield of oil. Lower concentrations gave lower oil yields with a putrid odour, while a higher formic acid concentration also gave lower and acid tainted yield.

Acid value

The acid value increased correspondingly with the increase in formic acid added. With the addition of 0.5% formic acid, the acid value was 0.59, whereas with 4% formic acid the acid value increased to 0.97. Such an increase may be due to the emission of acid into the oil during the separation of water-residue process; otherwise, high acid concentration might have caused oil hydrolysis with a concomitant increase in free fatty acids.

Iodine value

The iodine value of the acid ensilaged shark liver oil varied between 239 to 254. The results showed that there was no direct correlation between the volume of formic acid added and the iodine value. The results might, however, indicate that the high iodine value was due to the high content of double bonds (unsaturated fatty acids).

Specific gravity

The specific gravity of the acid ensilaged oil extract varied between 0.85 to 0.88 g/ml. There was evidence that the specific gravity of the oil extract increased in line with the increased formic acid concentration. The specific gravity of liver oil using 0.5 and 4% formic acid for ensiling process was found to be 0.85 and 0.88 g/ml, respectively. It is postulated that this was due to triglyceride hydrolysis leading to the formation of smaller molecules of fatty acids and glycerol.

Squalene identification

The physico-chemical properties of squalene obtained from this study is summarised in the following table:

Table 2. Physio-chemical properties of isolated squalene compared to the squalene standard

Parameter	Squalene standard	Isolated squalene
Specific gravity	0.8436 g/ml (26°C)	0.8498 g/ml (26°C)
Refractory index	1.4997 (25°C)	1.4999 (25°C)
Boiling temperature	241 - 242°C	242 - 244°C
Solubility in n-hexane	Completely soluble	Completely soluble
Rf (TLC)	0.57	0.53
Retention time (GC)	71.2 min.	71.28 min.

It appears that the specific gravity of isolated squalene is slightly higher than the squalene standard. Such a difference indicates the presence of impurities in the squalene isolate, especially glycerol or fatty acids.

Refractometry index, boiling temperature and chromatography tests support this claim. The refractory index of the squalene standard and squalene isolate is 1.4999 and 1.4997, whereas the boiling temperature is 242 - 244°C and 241 - 242°C respectively. The Rf of the squalene standard on silica gel plate with n-hexane eluent is 0.57 while that of the squalene isolate is 0.53. Gas chromatography analysis shows that the retention time for the squalene standard and the squalene isolate is 71.20 and 71.28 min., respectively. The differences are obviously due to the difference in their purity, as the squalene isolate still contains other substances.

Squalene yield and concentration

The study showed that out of 5 g of shark liver oil sample, about 3.1 - 3.4 g of squalene could be isolated (approximately 70%). This squalene extract was found to contain about 90 - 93% pure squalene. With repeated trials, it was found that the maximum oil and squalene yield under this study was 62 - 68% and 90 - 93%, respectively.

Conclusion

The following conclusions are drawn from this study:

- The optimum concentration of formic acid for extraction of shark liver oil using acid ensiling method is between 1.5 - 2.5%.
- The amount of formic acid added for ensiling process has no significant effect on the squalene yield.
- High iodine value of the shark liver oil sample indicates the presence of a number of double bonds.
- The squalene content of the *C. squamosus* liver oil sample is about 68%.

Based on the above findings, the following are important points which need to be taken into consideration for future studies on squalene isolation:

- It is recommemded to maintain low temperature during separation of the solvent and eluent (probably using rotary evaporator) from squalene, since squalene appears to be susceptible to oxidation especially under high temperatures.
- It was found that the stationary phase of column chromatography used in this study is not satisfactory. It is therefore recommended to find a more suitable stationary phase for future studies.

References

Abdurrahman and Saleh, M. 1976. Percobaan Cara-cara Pengawetan Hati Ikan Cucut dan Cara-cara Ekstraksi Minyaknya. J. Penelitian Teknologi Hasil Perikanan. BPPL, Jakarta, 24 - 26:

Broddy, Y. 1965. Fishery By-product Technology. The AVI Publishing Co. Inc., 439.

Kreuzer, R. and Ahmed 1976. Shark Utilisation and Marketing. Food and Agriculture Organisation of the United Nations, Rome.

Mustafa, A. 1984. New deep-sea spiny dogfish resources of Andaman. ICLARM Quarterly, Aberdeen Manzil, India: 8.

Stansby, M. 1965. Fish Oils. The AVI Publishing Co. Inc., 439.

Sunarya, M. 1987. Extraction and storage stability of nutritionally important compounds of shark liver oil. Ph.D. Thesis. Humberside College of Higher Education, Grimsby, United Kingdom.

Yunizal 1983. Studi Cara-cara Ekstraksi Minyak Hati Ikan Cucut. J. Penelitian Teknologi Hasil Perikanan, BPTP, Jakarta (22):35-37.

Yunizal and Nasran, S. 1983. Ekstraksi Minyak Hati Ikan Cucut dengan Proses Silase Asama. J. Penelitian Teknologi Hasil Perikanan, BPTP, Jakarta (26):24-26.

MICROBIAL ENSILAGE OF TRASH FISH FOR ANIMAL FEEDS

Crisanto S. Lopez, Jr.
Office of Research, West Visayas State University, La Paz, Iloilo City, Philippines

Summary

Microbial ensilage of trash fish through anaerobic fermentation using molasses and a starter culture of *Lactobacillus plantarum* was studied at ambient temperature. Fish silage was successfully produced in the laboratory after 21 days of fermentation with 15% molasses. From an initial crude protein of 8.87% in all treatments, the crude protein content of the treatment inoculated with *L. plantarum* rose to 14.09% (wet weight basis) as compared to 12.89% of the uninoculated treatment. The pH of the different treatments lowered from 6.0 to 4.0 in all treatments except in the control which rapidly spoiled during the fermentation.

For ease of storage, the use of binding materials for the silage was studied. Coconut coir and rice straw were considered because of their abundance and good absorption capacity. The milled binder was added at a ratio of 150 g/kg of the silage. At this level, the product can easily be dried and the fiber content is within the maximum allowable limit for animal feeds.

Introduction

When the parts of fish are converted into products of commercial value, where no wastage is allowed, optimum utilisation is attained. This is hardly achieved in the tropical fishing industries due to transport difficulties, lack of ice and cold storage facilities, inadequate processing and lack of technical knowledge on the total utilisation of the fish and its by-products. For these reasons, considerable amounts of fish are wasted or even discarded at the time of catching.

The Philippines is no exception to this. The total fish production in 1977 was about 1.5 M tons of which about 40% was by-catch (Tapiador, 1979). The by-catch is small fish and other organisms which are at times dumped overboard since the fishermen reserve chilled space for storage of possible catch later during the trip.

Attempts are made to preserve the fish either by drying or other means, however, inadequate facilities often result in poor quality products which if subsequently dumped, contribute further to the already burgeoning problem of pollution.

Slightly spoiled fish are processed into minor products like "patis" and "bagoong". These are fermented foods prepared from fish with the use of salt. Some by-catch is distributed to fish meal processors. Ideal fish meal processing however, is a capital intensive, much more tedious process. The fish is boiled or steamed, pressed and dried. Almost 40% of the protein is lost when the residual liquid is discarded during the processing (Kompiang, 1979). Improper drying makes it susceptible to the growth of moulds thereby producing a poor quality product in terms of protein content and may even pose a hazard to animals when used in their feed.

An alternative process of technology in trash fish utilisation is needed for an added value product. Microbial ensilation of trash fish is one simple alternative and has many advantages (Summer, 1976). The technology is simple and requires little capital even for large scale production. Much more, the process is suitable under tropical conditions.

Objectives

This study aims to:

1. Utilise trash fish directly by ensilation through microbial fermentation.
2. Develop a low-level technology for fish ensilation where the process can even be carried out in various fis ports in the Philippines.
3. Solve the problem of storage and transportation of fish silage by determining the ideal binding material fo liquid product so a powdery or paste-like consistency can be obtained.
4. Help alleviate protein supplement scarcity in animal feeds through the use of fish silage.

Materials and methods

The laboratory trial consisted of the following treatments: treatment A, control (pure trash fish); treament B, trash fish 15% molasses while treatment C consisted of trash fish plus 15% molasses and inoculated with 5% starter culture of *Lactobaci plantarum*.

The ungutted fish (*Sardinella fimbriata*) was weighed and distributed among the different treatments using 500 capacity Erlenmeyer flasks as containers. To treatment A, 250 g of fish was added. To treatment B, 250 g of fish and 15% molas (by weight) was added. Treatment C contained 250 g of fish plus 15% molasses and was then inoculated with 5% starter o *plantarum* in broth culture. Each treatment was replicated 5 times. The mouth of the flasks were covered tightly with plastic she and secured with rubber bands. The whole set-up was arranged in the laboratory in a completely randomised design (CRD). T incubation temperature was between 30 to 35°C. The incubation period lasted for 21 days during which the pH and the cr protein content of the different treatments were monitored periodically.

Results and discussion

The average pH values of the different treatments are presented in Table 1.

Table 1. Average pH values of the different treatments during the period of fermentation (21 days)

Treatment	Days					
	0	3	7	10	15	21
B	6.20	4.49	4.25	4.16	4.20	4.23
C	6.20	4.18	4.10	4.08	3.99	4.01
Statistical significance	NS	**	**	**	**	**

NS -Not significant

** - Significant at 1% level

All the treatments were at pH 6 at the time of the preparation of the silage. After 3 days of fermentation, except for treatmer A (control) which became spoiled, the pH of both the treatments dropped to 4 and remained at such a value up to the last day o fermentation. Statistical analysis of the data revealed a highly significant difference between the pH values of the inoculated an uninoculated treatments from the 3rd day up to the last day of fermentation.

One of the reasons for the drop in pH is that enough acid was produced during the fermentation, thus ensuring a pH at a level ideal for preservation of the product. Fish contain a variety of microorganisms and to have a successful ensilation, a suitable carbohydrate must be added. The drop in pH is brought about by the fermentation of the available carbohydrate. This is due to the natural microflora of the fish and the resulting product is mainly lactic acid (Kompiang et al., 1977). As shown in treatment C however, the inoculation of *Lactobacillus* in addition to the natural microflora of the fish brought a more rapid drop in the pH of the product. In this particular case, molasses served as a source of carbohydrate which speeded up the reaction. This may explain the spoilage of treatment A. This treatment did not have enough available carbohydrate upon which the microorganisms could act for conversion into an acidic product, a condition necessary for product preservation.

Visual observations revealed that the fish started to liquefy on the 7th day of fermentation. Proteolytic enzymes inherent in the fish contributed to the hydrolysis by breaking the peptides (Rao, 1965), thus liquefaction of the fish is attained.

Of the different treatments made, the crude protein of treatment C was higher compared to that of treatment B after 21 days of fermentation. Table 2 shows that there is a highly significant difference in the crude protein content of the different treatments from 3 days of fermentation up to 21 days. There was a significant rise in the crude protein of treatment C from 8.87 to 14.09% (wet weight basis) after the fermentation period against that of treatment B which is 12.89%.

Table 2. Per cent crude protein content of the different treatments during the period of fermentation (wet weight basis)

Treatment	Days					
	0	3	7	10	15	21
B	8.87	9.02	10.51	11.30	12.51	12.90
C	8.87	9.12	10.92	12.01	13.01	14.09
Statistical significance	NS	**	**	**	**	**

NS Not significant

** Significant at 1% level

Based on the results of the study, large scale trials were done using big plastic containers and large quantities of trash fish obtained from a fish landing area in Muelle Loney, Iloilo City, Philippines. The mixed ungutted fish was weighed and 15% molasses was added to it. To the mixture, 5% starter culture of *Lactobacillus plantarum* was added as inoculum. The starter culture was grown in boiled rice in a plastic bag for about 3 days prior to the inoculation of the fish preparation. The pH and the crude protein were likewise monitored for 21 days. Two locally available agricultural waste materials were considered as binding materials for the liquid product. These are the rice straw and coconut coir. These materials were dried and milled and mixed with the fermented product. The milled binders were added at a ratio of 150 g per kg of the fish silage. At this level, the products were easily dried and the crude protein content was analysed at 35% while the crude fiber was about 20%.

References

Kompiang, I.P., Arifudin, R. and Roa, J. 1977. Nutritional value of ensilaged by-catch fish from Indonesian shrimp trawlers. In: Proceedings of the Torry Jubilee Conference, Aberdeen, Torry Research Station.

Kompiang, I.P. 1979. Utilisation of trash fish and wastes in Indonesia. A paper presented in a workshop on Bio-conversion of Ligno-cellulosic and Carbohydrate Residue in Rural Communities. Indonesia, 1979.

Rao, P.D. 1965. Ensilage of fish by microbial fermentation. Fish News Int., 4(3). 283-286.

Summer, J. 1976. IPFC Working Party on Fish Technology and Marketing. Colombo, Sri Lanka (FT/74/4).

Tapiador, P. 1979. Needs and requirements of fisheries extension services on fish silage development. Paper presented ASEAN Seminar Workshop on Fisheries Extension. Metro Manila, Philippines.